GRAIN DEVELOPMENT IN WHEAT IN RELATION TO PHOSPHORUS

by

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PAGE 128 MISSING
TEXT COMPLETE

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GRAIN DEVELOPMENT IN RELATION TO PHOSPHORUS

I have attended to the matters raised by the three referees of my Ph.D. Thesis. Typographical errors have been corrected and a number of additions and corrections made as requested.

I do not agree with some comments made by individual referees and therefore have not made some changes which were suggested. My reasons for not agreeing to make these changes are as follows.

Report by Referee A (8 pages long)

In several cases (e.g. page 42) the terms used are consistent with standard and accepted terminology and layout in this field of research.

Some problems with interpretation were due to minor errors in sentence structure or to taking sentences out of context relative to previous sentences(e.g. page 47).On page 49 my wording is based on the literature cited in that section and is therefore acceptable.

Where I did not specify phosphorus concentration or amount (e.g. pages 20,41) either there was no need to be specific, or the reference cited did not state which value.

On page 95 the plant names were used as adjectives and not as formal plant names.

The caption to Plate 1 includes a description of the peak due to copper for clarity and completeness. The caption to Fig.8.5 is similar to others in the literature on this topic. The peaks are not named in the caption because they must be considered with the qualifications discussed in the text.

Table App.6.10 does not contain grain %P data because the grains were analysed as shown in Table 3.6 and so are not strictly comparabl

Report by Referee B (4 pages long)

I have reexamined my interpretation of Fig.3.8 in the light of the references quoted by the examiner and believe that one reference,which reports work on detached leaves,fails to contradict my argument , while the other paper supports my argument. I have changed the term senescence to death on page 52 to reduce any confusion on this part of the thesis.

The low P plants referred to on page 114 need not be as deficient as suggested by the examiner to be beyond the limit of detection of the NMR technique being reported.

It does not follow that there is little point changing a small pool of phosphorus within the plant - it may be the most active pool. This section on potentially desireable plant characters should be read with the view that the features , if changed , "..could lead to more efficient utilization .."

I have discussed the changes with the Staff of the Botany Department.

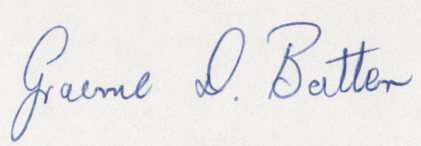
Graeme D. Batten

Graeme D. Batten
26.10.1984

DECLARATION

I hereby certify that the work presented in this thesis is my own and has not been submitted for any degree or diploma unless otherwise acknowledged. The photographs and EDX images in Chapter 7 were made by Dr J.N.A. Lott, at CSIRO Division of Plant Industry while on leave from McMaster University, Hamilton, Ontario, Canada. The studies reported in Chapter 8 were instigated by me and conducted with Dr J.I.L. Morison, CSIRO Division of Plant Industry, Canberra, and Dr M.D. Fenn, John Curtin School of Medical Research, Medical Chemistry Department, A.N.U.

Material which has been published or presented for any degree is referenced. Other assistance given during the period of experimental work and for the preparation of the thesis is acknowledged.



Graeme Douglas Batten

ACKNOWLEDGEMENTS

I am grateful to the New South Wales Department of Agriculture for leave to undertake full-time studies. Financially I was also supported by a Commonwealth Postgraduate Research Award supplemented by an allowance from the Australian National University.

I owe much to my family for sticking with me during this project. In addition to taking leave from her career my wife Lyn kept our home together and, with Marcel, assisted with preparing countless samples.

During the project I was fortunate to have access to excellent facilities in the University Departments of Botany, Forestry, Medical Chemistry and Computer Sciences and in the CSIRO Division of Plant Industry. Throughout I was supported and encouraged by two experienced supervisors, Dr Mervyn J. Aston in the Botany Department and Dr Ian F. Wardlaw in the Plant Physiology group of CSIRO. In addition Dr Graham Farquhar of the Research School of Biological Sciences acted as an advisor. Mrs Erina Aston and Mrs Judy Wardlaw kindly extended much hospitality to me and my family.

Many colleagues also assisted me during the project. Dr J.L. Davidson, Dr M.A. Khan, Mr B.J. Scott, Mr K.J. Symes and Mrs M. Bush provided seed. Mr Iain Dawson, Phytotron Biologist, kindly provided space for experiments and Mr Steve Dillon, Mr Peter Munibi, Mr Gary Rosser and Mrs Marie Thompson assisted with watering and general care of plants in the Phytotron. Mr Dennis Porritt, Mr Gary Dinnerville and Mr Leo Trujillo of the Phytotron Workshop set up and maintained the equipment which I used.

I thank Mr Cliff Irving for assistance with initial phytate analyses, Mr Bruce Bennett for maintaining the autoanalyser in excellent working order, Dr Grahame Chilvers for helping me check for V.A. mycorrhiza, Mr Ross Cunningham for advice on statistical analyses and using Genstat, and my colleagues within the University and CSIRO for stimulating discussions and help with equipment.

Mr Roger Mummery, Mr Terry Murphy and Mr Max Minius assisted by preparing data for Chapter 8 and samples for Chapter 7.

GRAIN DEVELOPMENT IN WHEAT IN RELATION TO PHOSPHORUS

SUMMARY

During the last 100 years plant breeders have effected considerable increases in crop yields and in doing this have unconsciously improved the utilization of phosphorus. The cost of phosphate fertilizers is still, however, a significant expenditure to cereal producers and attempts to further improve the efficiency of utilization of phosphorus must be sought. The studies reported in this thesis were conducted to test the hypothesis that in modern wheats there is still room for improved phosphorus efficiency in relation to grain production (i.e. grain phosphorus concentration).

Wheat plants were grown in sand under controlled environmental conditions with different phosphorus supply regimes and examined mainly during the grain development phase in order to obtain a better understanding of the utilization of phosphorus and to find mechanisms which could be used to screen for genotypes with greater efficiency.

Two experimental systems (infusion with D+ mannose and nuclear magnetic resonance spectroscopy) which may facilitate direct manipulation and measurement of inorganic phosphorus within cellular compartments of whole leaf samples respectively were also investigated.

The wheat used in most experiments was Triticum aestivum L. (cv. Kite) and comparisons were made between the control (1 mM phosphorus supplied daily) and a low phosphorus regime (0.25 mM P supplied only during the first 12 to 20 days after planting).

The flag leaf area, flag leaf photosynthetic rate per unit leaf area (at anthesis), grains per ear of the main culm, and final grain dry weight were reduced to 60-65% of the control values by the low P regime. Grain dry weight differences could not be accounted for by the number of endosperm cells per grain.

The vegetative tissues of control plants remained green until after the grains had matured and the glumes had lost chlorophyll (i.e. senesced). Only about 21% of the phosphorus in the grain was derived from other plant parts because uptake by the roots continued throughout grain development. In contrast to this low P plants senesced rapidly as phosphorus was exported from older to younger tissues and, ultimately, to the grain. As much as 89% of the phosphorus in the grain was obtained by retranslocation from other tissues during the grain development period, and 12% of the phosphorus in the grain could have been drawn from the flag leaf blade. (The actual amounts varied with the temperature used to grow the plants). The flag leaf blade was completely senesced when the grains were only 60% of their final dry weight.

During grain development phosphorus in the flag leaf blade was mobilized from soluble ester, lipid and residue fractions but the soluble phosphorus concentration tended to be maintained. Senescence (loss of chlorophyll) was therefore associated with a general decline in all phosphorus fractions which precipitated the loss of essential nitrogenous compounds.

The low P plants in this work did not accumulate phytate in the grain until the final stage of maturity. Although the grains contained only 20 mM inorganic plus ester phosphate, compared to 30 or over in

control grains, these did not respond to late applications of phosphorus.

Phosphorus applied via the roots during grain development raised grain phosphorus concentrations and produced limited (< 6%) increases in grain yield. Foliar applications of phosphorus delayed senescence but raised the grain phosphorus concentration without raising grain dry weight. This was interpreted as evidence that even low P plants export more phosphorus to the grain than is required for grain growth. But even if this phosphorus was retained in the vegetative tissues it may be of limited value because it was found that foliar applications which maintained leaf greenness failed to promote photosynthesis sufficiently to promote grain development. This suggests that the factors which control final grain size (rate of development and duration) are established early in the life of the plant and cannot be overruled by late foliar phosphorus treatments.

The redistribution of carbon and phosphorus from the flag leaf blade were examined using $^{14}CO_2$ and $^{32}P_i$. In control plants labelled during the mid grain development phase 80% of the phosphorus exported over 24 hours was deposited in the grain. The proportion of the exported phosphorus deposited in the grain of low P plants in 24 hours was only 30%. In the early and late phases of grain development less phosphorus and carbon were deposited in the grain in the 24 hours after labelling. The ratio of carbon:phosphorus deposition in the grain was greater in low P plants especially during the early grain development phase. At the end of a seven day period of translocation 75% of the phosphorus exported from the flag leaf of low P plants was found in the grain. In low P plants retranslocated phosphorus was largely retained in the small inorganic pool and it is suggested that phosphorus is exported from the flag leaf faster than

it is required by the grain in the short term and is stored largely as inorganic phosphorus in the stem in preference to entering the grain where it will be stored as phytate.

The genus Triticum evolved about 10,000 years ago and contains wheats of three ploidy levels. Twenty genotypes were examined to assess the effects of evolution on grain phosphorus. Increases in yield per ear associated with increasing ploidy were negatively related to grain phosphorus concentration, but the more reliable plant character was harvest index which had a curvilinear relationship with grain phosphorus concentration. Within the bread wheat genotypes, grain phosphorus in low P plants varied by 30% at a harvest index of 38%. This indicates that there is still ample variation within modern wheats to encourage selection for genotypes which produce high grain yields with lower grain phosphorus concentrations. The use of traditional indirect breeding methods and direct screening techniques are discussed.

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CHAPTER 1

GENERAL INTRODUCTION AND REVIEW OF GRAIN DEVELOPMENT IN RELATION TO PHOSPHORUS

1.1 GENERAL INTRODUCTION

By the year 2000 AD the world population is expected to exceed 6,250,000,000 and it will be necessary to increase the total global production of food by 50 to 60% (over the 1980 level) to meet the needs of those people. Much of this increase is likely to occur in the present agricultural arid areas. Then, as now, fertilizers will be a key to this production (FAO, 1981).

The world consumption of phosphorus fertilizer in 1980/81 was 15,031,000 tonnes of elemental P and over 78% of this was used in developed countries (FAO, 1981). In Australia phosphorus is the dominant form of fertilizer used and in 1980/81 the consumption of phosphorus, nitrogen and potassium were in the proportions 1.94:1:0.69 compared for example to 0.21:1:0.43 in North America (FAO, 1982). Consequently, the energy in phosphorus fertilizer makes up 13.4% of the gross energy input for food production in Australia compared to only 1.5% in the USA and Holland (Gifford, 1976). The consumption of phosphorus has fluctuated during the past two decades. Between 1966/67 and 1970/71, it declined from 410,000 to 325,000 tonnes P/annum; rose to 540,000 tonnes in 1973/74; declined dramatically to 200,000 tonnes in 1975/76; and has increased steadily to 350,000 tonnes in the early 1980's. About 40% of this phosphate is now used for the production of wheat and other cereal crops (Australian Bureau of Statistics, 1983). The oscillations in phosphate fertilizer use per hectare are closely linked to farmer cash operating surplus income per hectare rather than the price of the fertilizer (Gargett, 1983).

For Australian cereal growers the cost of phosphate fertilizer forms 20-25% of the total cost incurred in producing a crop (Colton,

1979). Lipsett and Dann (1983) draw attention to the real cost of exporting nutrients from Australia in wheat. But the problem of exporting nutrients is a matter for more general concern. Countries which export food, and most Western Societies, maintain food production by an "open ended" nutrient chain (Figure 1.1). Minerals mined in one region are modified to give readily available plant nutrients, transported, and added to agricultural land.

This is a wasteful system compared to that which has long been practiced in China, and other societies (McCalla and Plucknett, 1981; FAO, 1978). There nutrients are returned to agricultural soils as soon as possible. In time, there could be greater use of disposal by System II. Parr et al. (1978) reviewed environmental legislation which could terminate disposal of sewage by dumping and burning in the United States and a technology which could easily convert sewage into useful fertilizer.

But even if such changes are effected and reduce the demands on mineral resources there are sound economic reasons for seeking to improve the efficiency of utilization of nutrients by plants. Efficiency may be improved in terms of

yield per unit of fertilizer applied (1)

yield per unit of nutrient taken up (2)

or yield per unit of nutrient in the economically valuable tissues (3).

In view of the importance of cereals as staple foods and the costs of phosphate fertilizers, there is very little information on the utilization of phosphorus in terms of (2) and (3) above.

In this thesis the role of phosphorus is examined in relation to grain development in wheat. Nutrition-physiology studies are

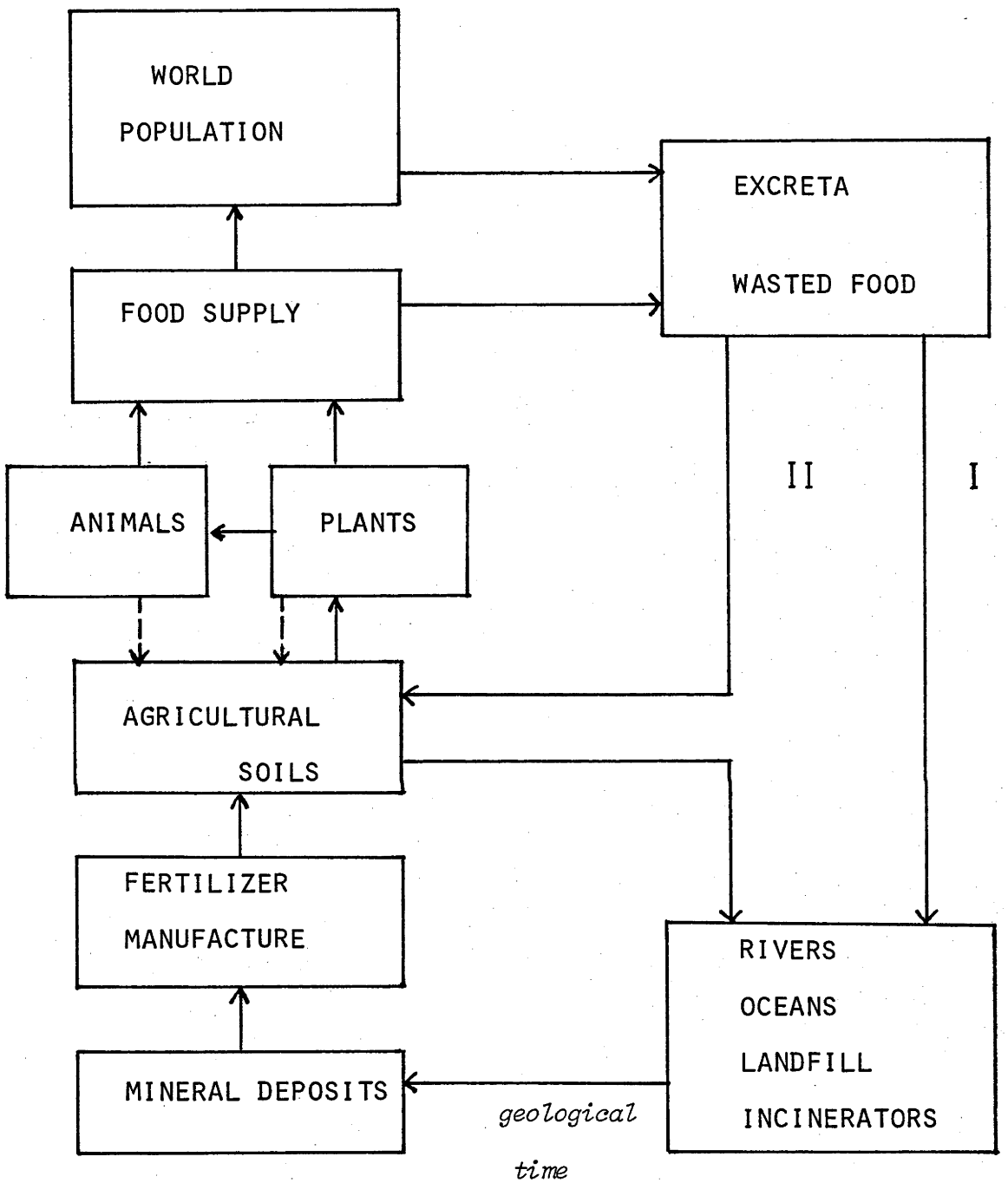

Figure 1.1 Diagram of two simplified global food chains. I. An "open ended" chain where nutrients are not returned directly to agricultural soils. II. A closed cycle where nutrients are reused in the short term.

reported which were conducted to test the hypothesis that modern wheat genotypes utilize phosphorus inefficiently.

In the literature review which follows "modern wheats" are placed in context. The central role of phosphorus for grain yield, arguments for thinking the efficiency of utilization of phosphorus can be improved, and the importance of phosphorus for grain quality are discussed.

1.2 THE EVOLUTION OF WHEAT

1.2.1 The evolution of modern wheats

Wheats are believed to have originated and evolved in the Middle East Region some 10,000 years ago. Archaeological evidence has been obtained in Egypt, S. Jordan, Palestine, Israel, N. Syria, S.E. and S.W. Turkey, S. Greece, N.E. Iraq, S.W. Iran and Baluchistan Pakistan (Harlan, 1981).

The evolution and relationships of modern wheat have been reviewed by several authors including Percival (1921), who cited classifications by pre-Linnean botanists, Riley (1965), Bell (1965), and Mac Key (1966, 1977).

Mac Key (1966) proposed a revision of Triticum to include five species in three ploidy levels, while Mac Key subsequently (1977) proposed division into six species as shown in Table 1.1. This classification was based on the degree of interrelations and crossability between different types, their possibilities for introgression, and also other isolating aspects such as geographic distribution and agroecological specialization.

Bread wheats carry three sets of chromosomes in the A, B and D genomes. Genome A being traced to diploid wheat as progenitor, genome D to Aegilops squarrosa, but genome B could be only vaguely tied to the Sitopsis group of Aegilops (Mac Key, 1977).

Table 1.1 Subdivision of the genus Triticum (L.) Dum. according to genetic conceptions

Monococca FLAKSB. diploid; 2n = 14	Dicoccoidea FLAKSB. tetraploid; 2n = 28	Speltoidea FLASKSB. hexaploid; 2n = 42
T. monococcum L. einkorn wheat ssp. boeoticum (BOISS.) L. et L. wild einkorn wheat var. aegilopoides (LINK) MK var. thaoudar (REUT.) PERC. ssp. monococcum einkorn wheat	T. timopheevi ZHUK. zanduri wheat ssp. armeniacum (JAKUBZ.) MK wild zanduri wheat ssp. timopheevi zanduri wheat	T. zhukovskyi MEN. et ER. timon wheat
T. urartu TUM. zweikorn wheat	T. turgidum (L.) THELL. emmer wheat ssp. dicoccoides (KORN.) THELL. wild emmer wheat ssp. dicoccum (SCHRANK) THELL+ (true) emmer wheat ssp. georgicum (DEK. et MEN) MK kolchis wheat ssp. turgidum conv. turgidum Rivet wheat conv. durum (DESF.) MK * macaroni wheat conv. turanicum (JAKUBZ.) MK khorosen wheat conv. Polonicum (L.) MK Polish wheat ssp. carthlicum (NEVSKI) L. et L. dika wheat	T. aestivum (L.) THELL. dinkel wheat ssp. spelta (L.) THELL. spelt wheat ssp. macha (DEK. et MEN.) MK macha wheat spp. vulgare (VILL.) MK * bread wheat ssp. compactum (HOST) MK club wheat ssp. sphaerococcum (PERC.) MK Indian dwarf wheat

* commonly cultivated today .

\+ cultivated today in some countries (Perrino and Hammer,1983).

It would be dangerous to draw conclusions as to the evolution of modern bread wheat from an examination of relatively few representatives of each ploidy level. Over the 10,000 years since the original combination or series of combination events occurred (Harlan 1981), natural introgression, substitutions and mutations may have been differentially affected by climatic and edaphic variations, especially under cultivation. Within modern bread wheats for example, genotypes selected in the state of Ohio were found to be more tolerant of acid soils than genotypes selected in the neighbouring state of Indiana (Foy et al., 1974). The time period over which this change occurred is minute compared to that above. This is of particular interest in the context of the current work as it is seen as confirmation that at the specie, subspecie, convariety and cultivar levels wheat could have evolved with different degrees of efficiency in utilization of phosphorus.

1.2.2 Phosphorus changes associated with the evolution of wheat and wheat breeding

Percival (1921) noted that T. monococcum was resistant to "poor soils" but did not clarify this remark. No evidence has been cited to show if, with evolution, wheats have become more dependent on fertilizer or soil phosphate. Kamprath and Foy (1984) cite publications which suggest that plant genotypes within many species (sorghum, bean, cowpea, pearl millet, orchard grass, white clover, rice, Pennisetum americanum, tomato, corn, barley and wheat) differ widely in the uptake and use of phosphorus.

Of the few studies which are available on wheat positive relationships between uptake of phosphorus and plant growth and yield have been found (Palmer and Jessop, 1977). Jessop et al.

(1983) reported higher yields by semidwarf, compared to taller genotypes of wheat, when the uptake of phosphorus was low, but when uptake was higher differences between the two groups were less clear.

But, when evaluating the utilization of phosphorus, by cereals, to produce grain it is also essential to consider the deposition of phosphorus into the grain relative to the deposition of carbon (i.e. grain phosphorus concentration) and the consequences of removing phosphorus from vegetative tissues (Williams, 1948).

1.3 GRAIN FILLING AND THE ROLE OF PHOSPHORUS

1.3.1 Introduction

Assimilation of carbon, by the processes of photosynthesis, and translocation to and incorporation of assimilate into the grain constitute grain filling. Higher grain yields are believed to be attainable by increasing the rate and duration of photosynthesis (Austin, 1982), and the translocation of assimilates (Giaquinta, 1983). The role of phosphorus in photosynthesis, translocation and grain filling is examined below.

1.3.2 Phosphorus in vegetative tissues

In the shoots of cereals phosphorus usually forms less than 1% of the tissue dry weight (an exception is in necrotic leaves of plants grown with toxic levels of phosphorus; Bhatti and Loneragan, 1970a,b). This phosphorus may be divided into four chemical fractions, namely inorganic, lipid, ester and RNA/DNA or residue (Bieleski, 1968a).

Inorganic phosphorus (P_i), as a proportion of the total phosphorus, varies between species (e.g. Urtica dioica retained less

phosphorus in the inorganic form than did Deschampsia flexuosa; Nassery, 1970) and has been shown to increase with phosphorus supply in oats (Williams, 1948), barley (Chapin and Bieleski, 1982), legumes (Hart and Jessop, 1982), ryegrass (Dijkshoorn and Lampe, 1961) and tomato (Roux, 1966; Weste et al., 1974).

Phosphorus is intimately involved in the structure and metabolism of lipids, which have a variety of biological roles including storage of energy and the formation of membranes. Phospholipids are derived from either glycerol, a three-carbon alcohol, or sphingosine, a more complex (C_{30}) alcohol and are key constituents of membrane because they are able to form extensive bimolecular sheets with distinct permeability characteristics.

Ester phosphorus compounds (those present in the methanol-water component following the Bieleski, 1968a procedure) include C_3, C_5, C_6 etc. sugar phosphates and diphosphates, phosphoenol pyruvate, the purine and pyrimidine sugar mono, di- and tri-phosphate (ATP, UTP, GTP, CDP etc.), portions of the lipid phosphate pool, such as phosphoglyceric acid, phosphoryl ethanolamine, and phosphoryl choline.

RNA/DNA (residue) compounds contain phosphorus molecules which link the sugar molecules of nucleoside chains (Figure 1.2). The RNA:DNA phosphorus ratio in Spirodela is 5200:600 μmoles P (g fresh wt)$^{-1}$ (Bieleski, 1968a).

The proportion of P_i to total P has also been shown to decrease rapidly under phosphorus stress in most species examined e.g. Spirodela (Bieleski, 1968b), Stylosanthes hamata (Hart and Jessop, 1982), Sesame and pepper (Nassery et al., 1978). However, as Weste et al. (1974) demonstrated, not all tissues show similar changes in

phosphorus fractions. No studies of the phosphorus fractions in wheat have been cited.

Inorganic phosphorus is compartmentalised in the cell in the cytoplasm and the vacuole; designated as metabolic and non-metabolic pools (Bieleski, 1968b). The exchange of phosphorus between the vacuole and the cytoplasm occurs with a half time of more than 3 days (Bieleski, 1973) and the role of vacuolar phosphorus in higher plants requires further study. This in part depends on the development of suitable techniques. (This is examined in Chapter 8).

Phosphorus stress has been shown to lead to the degradation of phospholipids in Spirodela (Bieleski, 1968b), oats (Williams, 1948), tomato (Weste et al., 1974) and, to a lesser extent, in legumes (Hart and Jessop, 1982). The phosphorus esters are metabolically very active, especially in phosphorus deficient plants (Bieleski, 1968b) and are utilized in times of phosphorus stress, but at a slower rate than inorganic phosphorus, and apparently prior to the utilization of phospholipids or RNA phosphorus (Bieleski, 1968b; Weste et al., 1974). Under phosphorus stress, residue compounds are utilized to some extent in oats (Williams, 1948), Spirodela (Bieleski, 1968b), tomato (Weste et al., 1974), and in white clover but not in stylosanthes (Hart and Jessop, 1982).

In vegetative tissue the reported levels of phytate (the dominant form of phosphorus in the grain; 1.3.5) vary widely (2 to 84% of the total), due possibly to the use of less than reliable techniques (Playne, 1976) and there is a need for further work in this area. Polyphosphates, which accumulate in the roots of Banksia ornata (Jeffrey, 1964), apparently do not accumulate in other plants (Nassery, 1969).

The concentration of total phosphorus in wheat shoots declines with age (Boatwright and Haas, 1961; McLachlan, 1982). The evidence above, from various species, indicates that phosphorus from each of the major chemical fractions will decline in the vegetative tissue of wheat during grain filling.

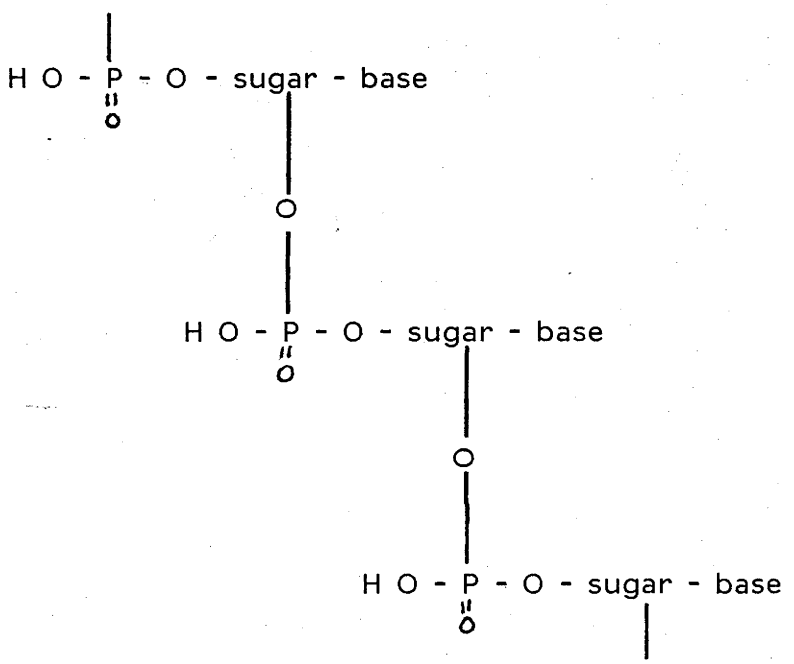

Figure 1.2 Nucleic acid where sugar refers to ribose or deoxyribose, and base refers to adenine, guanine (= Purine bases) uracil*, thymine** or cytosine (pyrimidine bases).
*in RNA only. **not in RNA.
- based on Bidwell (1979).

1.3.3 Photosynthesis and phosphorus

Phosphorus is involved at almost every step of photosynthesis.

On entering the chloroplast of a mesophyll cell CO_2 combines with the CO_2-acceptor ribulose bisphosphate to produce the three-carbon acid 3-phosphoglycerate[1]. CO_2 reacts with ribulose bisphosphate by the action of the unique enzyme ribulose bisphosphate carboxylase/oxygenase, now termed rubisco, which is the major soluble leaf protein (for review of the carboxylation and oxygenation activity of rubisco, see Lorimer, 1981). Phosphorus deficiency does not directly affect the carboxylase or oxygenase assay of rubisco (Kabaki and Tajima, 1982).

The reductive pentose phosphate pathway (= Benson-Calvin Cycle; Figure 1.3) generates phosphorylated sugars with C_3, C_4 up to C_8 carbon skeletons (Williams, 1980). These reactions proceed with energy input from ATP and NADPH (generated by photophosphorylation). The C_5 sugar phosphate ribulose bisphosphate is regenerated to accept more CO_2. The C_3 sugar phosphates (= triose phosphates) glyceraldehyde-3-phosphate and dihydroxyacetonephosphate are considered as the "product" from the RPPP cycle (Walker and Herold, 1977).

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[1] By this reaction wheat is defined as a C3 plant (as opposed to plants with C_4 biochemistry in which the CO_2 acceptor is phosphoenolpyruvate in the mesophyll cells, the initial product is a four carbon acid, and subsequent metabolism occurs in vascular bundle sheath cells (for details of C_4 anatomy and function see Laetsch, 1974; Hatch and Osmond, 1976; Edwards and Walker, 1980). Phosphorus has a central role in the metabolism of both C_3 and C_4 plants.

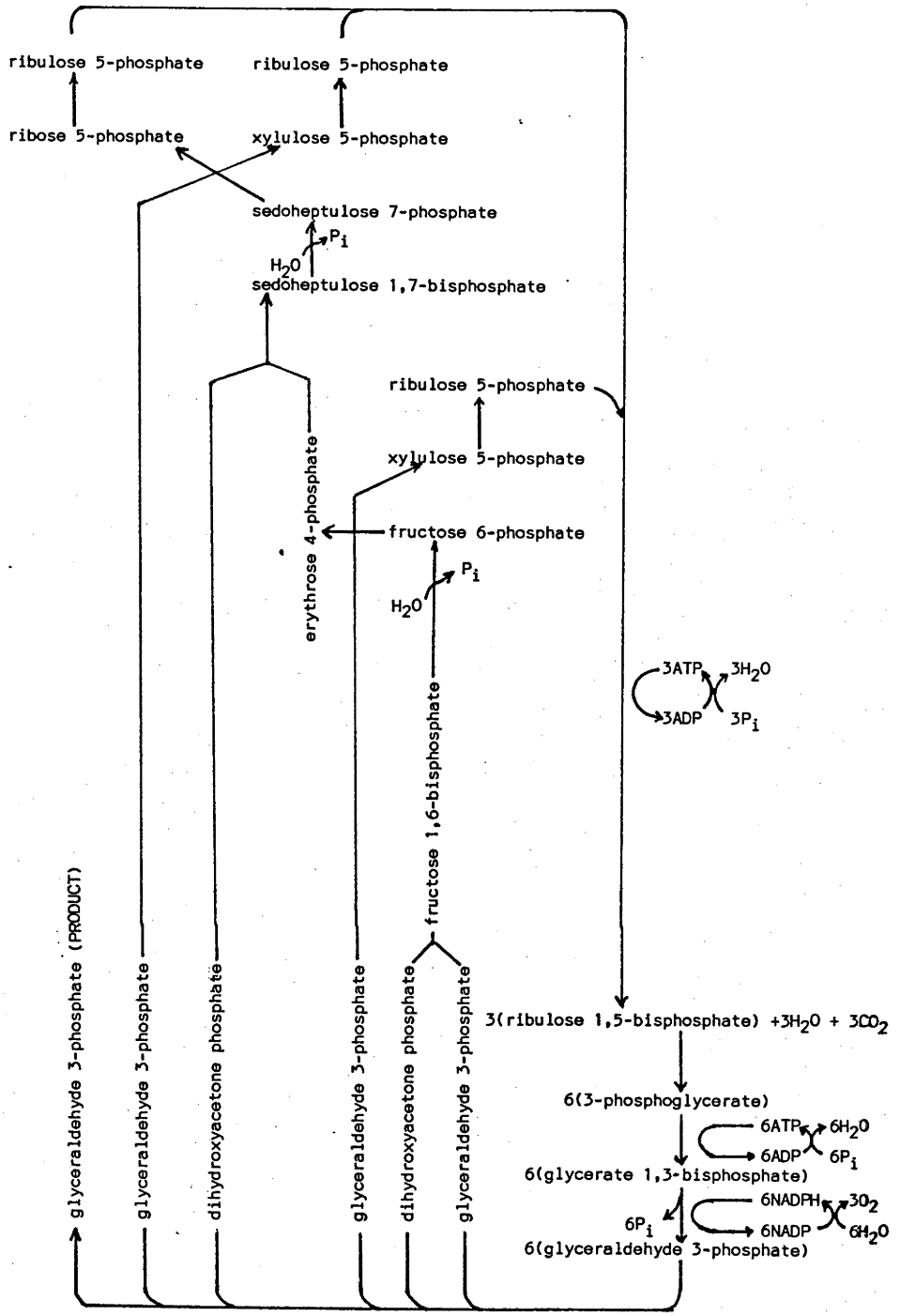


Figure 1.3 The reactions of the reductive pentose phosphate pathway (Benson-Calvin Cycle) plus associated photosynthetic phosphorylation, NADP reduction and oxygen evolution. In each revolution of this cycle, three molecules of CO_2 and one molecule of orthophosphate are taken up, the CO_2 acceptor is regenerated and one molecule of triose phosphate emerges as product. The cycle requires reducing power in the form of 6 molecules of NADPH and 9 molecules of ATP but it should be noted that 8 out of the 9 molecules of P_i consumed in photophosphorylation are released as each molecule of triose phosphate is formed.
- Walker and Herold (1977).

This "product" is either diverted to starch (although this does not normally accumulate in the leaves of wheat, King et al., 1967) or passes from the chloroplast into the cytosol. But export of triose phosphate only proceeds in exchange for inorganic phosphorus entering the chloroplast via the phosphate translocator (for a review of the chloroplast envelope see Heber and Heldt, 1981). Phosphate, phosphate esters, dicarboxylates and glucose are unable to pass passively through the inner envelope membrane of the chloroplast. ATP enters the chloroplast by counterexchange. Like pyrophosphate it is transported slowly.

Heber and Heldt (1981) state that phosphoenolpyruvate, glycerol-1-phosphate, erythrose-4-phosphate and ribose-5-phosphate are transported but the K_m values for these are at least an order of magnitude higher than for triose phosphates or 3-phosphoglycerate. There are also reports that hexose and pentose sugars are transported across the chloroplast envelope. For example glucose, the final product of hydrolytic starch degradation, is released from the chloroplast by the glucose translocator (Heber and Heldt, 1981). Repka, et al., (1981) suggested that starch may be released directly following partial lysis of the chloroplast membrane in the normal course of grain formation and filling in wheat. This report is contrary to the finding of King et al. (1967) and, therefore, requires confirmation.

Normally five sixths of the triose phosphate is used to regenerate ribulose bisphosphate (Figure 1.3). Superoptimal levels of inorganic phosphorus result in excessive export of triose phosphate from the chloroplast and a breakdown of the reductive pentose phosphate cycle (Heber and Heldt, 1981). Suboptimal levels of

inorganic phosphorus reduce the exchange of triose phosphate out of the chloroplast and favour the synthesis of starch (Giaquinta,(1980a) via activation of ADP glucose pyrophosphorylase (Herold and Walker, 1980).

In the cytosol triose phosphate undergoes aldol condensation to yield fructose-1,6-bis phosphate which is then dephosphorylated to fructose 6-phosphate and eventually UDPG and pyrophosphate, then sucrose phosphate and sucrose as shown in Figure 1.4 from Giaquinta (1980a).

These reactions maintain the level of inorganic phosphorus in the cytosol. This level of inorganic phosphorus for optimum CO_2 fixation by chloroplasts of wheat and other species in vitro was found to be in the region of 0.2 to 0.5 mM by Heldt et al., (1977) and Edwards et al., (1978). But Usuda and Edwards (1982) showed that this concentration of phosphorus was optimal for triose phosphate production under high levels (10 mM) of bicarbonate. At 0.3 mM bicarbonate, glycolate was the main product and > 0.025 mM inorganic phosphorus gave optimal photosynthesis. An increase in cytoplasmic inorganic phosphorus stimulates the export of triose phosphate from the chloroplast and hence the synthesis of additional sucrose. Comparisons of optimum phosphorus levels between genotypes are not available.

Starch synthesis is also favoured by a feed-back effect of high sucrose concentrations on sucrose phosphate synthetase. This could be due to a reduced transport of sucrose in the phloem e.g. following degraining (King et al., 1967) or reduced transfer to the vacuole.

At the whole plant level phosphorus controls plant growth but as Cartwright et al. (1974) stated "it is often difficult to separate the direct and indirect effects of nutrient stress". Natr (1972) reviewed

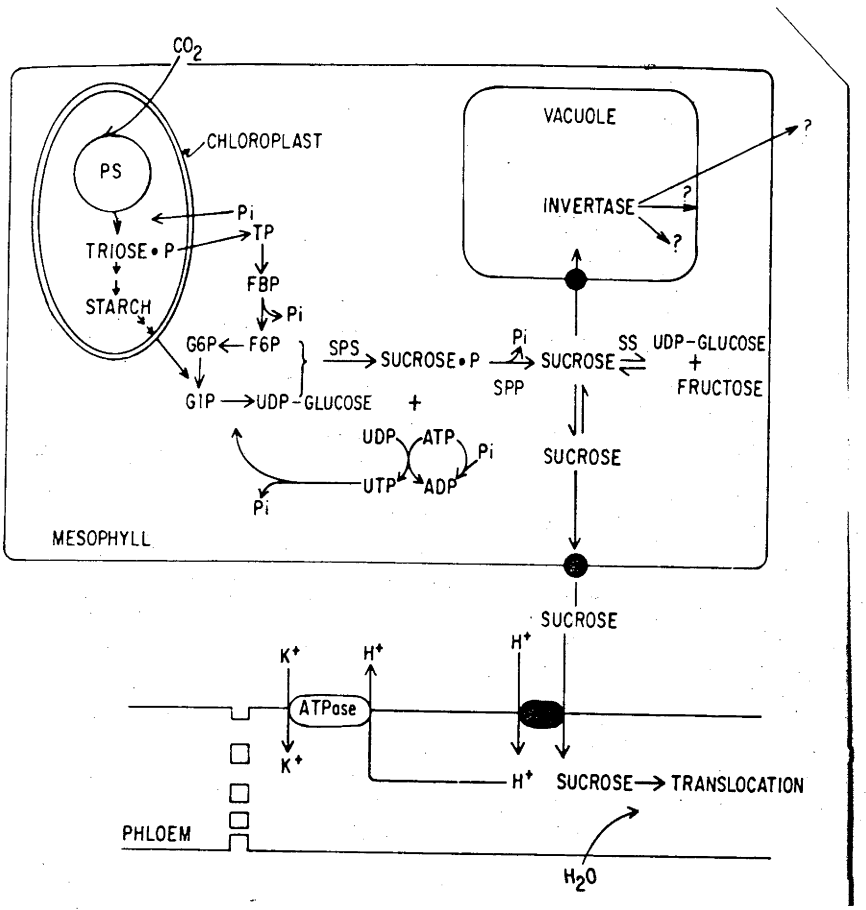


Figure 1.4

Summary of mesophyll–phloem interactions in relation to assimilate translocation. Possible sites of regulation include: (1) sucrose–starch conversions; (2) partitioning between transport and nontransport sucrose pools; (3) sucrose transport into and hydrolysis within the vacuole; (4) sucrose efflux from the mesophyll; and (5) sucrose entry into the phloem. TP, triose phosphate; FBP, fructose-1,6-bis(phosphate); F6P, 6-phosphate; fructose; G6P, glucose 6-phosphate; G1P, glucose 1-phosphate; SPS, sucrose phosphate synthatase; SPP, sucrose phosphate phosphatase; SS, sucrose synthase.

Giaquinta (1980a).

the effects of minerals on photosynthesis in higher plants. He cited reports of phosphorus deficiency increasing photosynthesis in barley and vegetables; causing little or no change in beans, soybeans, barley and Spirodela plants and causing strong inhibition in young barley, oat, tomato, beet, spinach and subterranean clover plants. Thus, no generalization can be made. There is evidence that the lack of an effect on photosynthesis is associated with the greater inhibition of leaf growth than chlorophyll formation, or chlorophyll present, per unit leaf area. Osman et al. (1977) also stated that the largest effects of major nutrients was on leaf area. Natr (1972) concluded that phosphorus deficiency could possibly affect photosynthesis by limiting the ATP content in leaves. This has been confirmed by the work of Walker and Robinson (1978) and Badger et al. (1983) who argue that a low supply of inorganic phosphorus can reduce the ATP/ADP ratio and hence reduce the regeneration of ribulose bisphosphate.

The reduced rate of photosynthesis under a low phosphorus supply has initially been associated with a high mesophyll resistance, but with continued phosphorus stress stomatal resistance appears to play a more important role (Terry and Ulrich, 1973; Cartwright et al., 1974; Longstreth and Nobel, 1980; sugar beet, wheat and cotton respectively). There is probably no direct link between plant phosphorus status and stomatal aperture. Farquhar and Sharkey (1982) argued that plants under other normal or stress conditions regulate stomata aperature in accordance with internal CO_2 concentration to optimize photosynthesis and minimize water loss.

Photosynthesis by the grain, the pericarp of which contains chlorophyll, may make a significant contribution to the total grain

weight where this is small as in ancient wheats (Evans and Dunstone, 1970), but would contribute relatively less in modern wheats which have large grains and utilize assimilate from the flag leaf, and peduncle as well as the ear structures (Figure 1.5). Quantititative estimations of photosynthesis by grains are difficult because of the shape of the grain (area exposed to light), the high respiration rate, and possibly, the physical act of removal from the ear (Wardlaw et al., 1980). Olegbemi et al. (1976) found that awnless ears contributed about 10% to the photosynthesis of the organs above the node of the penultimate leaf and awns increased this to about 18%. In bread wheats, 80 to 90% of the soluble carbohydrate in the mature grain is that assimilated, by photosynthesis, and transferred during grain development. The remainder is obtained from stem reserves built up prior to anthesis (Bidinger et al., 1977; Austin et al., 1977; Thorne, 1982).

The transfer or translocation of assimilate from the mesophyll cells to the grain is associated with the transfer of phosphorus (Marshall and Wardlaw, 1973). This has important implications to the overall efficiency of utilization of phosphorus.

1.3.4 Translocation of sucrose and phosphorus

Translocation is generally considered to have three phases, namely phloem loading, long distance transport, and phloem unloading.

The dominant route for the translocation of nutrients and assimilate to the grain is the "direct" connections of the phloem sieve elements. Some nutrients (including inorganic phosphorus) may however circulate via the phloem to the roots, where they are

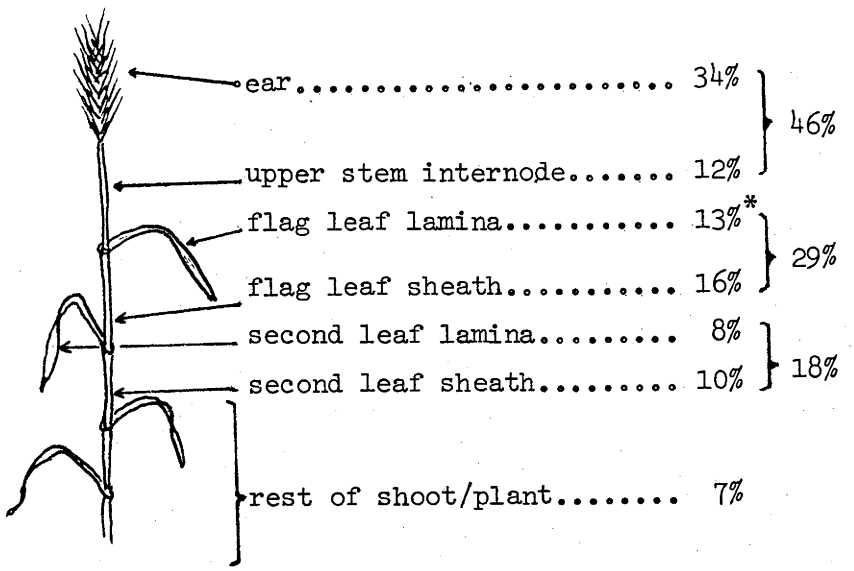


Figure 1.5 Relative contribution of assimilating organs to yield of grain estimated by defoliation and shielding experiments. Contribution of leaf sheaths estimated by interpolation (after Boonstra, 1937; Mac Key, 1968/69).
*Wardlaw et al. (1965) found that the flag leaf contribution was 14 to 28%. However, even this figure may underestimate the real contribution of the flag leaf. On removing the flag leaf there may be a compensatory increase in the assimilation by lower leaves due to increased light incidence and relatively greater demand for these assimilates by the "sink" (King et al., 1967).

transferred to the xylem and reach the grain in the transpiration stream or by re-entering the phloem in the stem (Pate, 1975). Phloem to xylem transfer also occurs in the stem and Martin (1982) reported that the translocation of nitrogen, phosphorus and magnesium to the ear of wheat was not reduced by interrupting the phloem in the stem of whole or detached culms (3 leaves). Movement of calcium was reduced and movement of potassium almost completely inhibited by this treatment.

Leaf morphology can be important in relation to both photosynthesis and vein loading.

Light saturated photosynthetic rates are negatively correlated with mesophyll cell size in Lolium perenne (Wilson and Cooper, 1969) and the spacing of the vascular bundles in wheat (Khan and Tsunoda, 1971; Austin et al., 1982), but positively correlated with the surface area of mesophyll cells directly exposed to air-filled space per unit leaf area (Parker and Ford, 1982). In C_3 grasses four to seven mesophyll cells may distance chloroplasts from the phloem (Wardlaw, 1980). Kuo et al. (1974) and Altus and Canney (1982) discuss the anatomy and loading of assimilate in wheat. The main flux of sugar from a wheat leaf occurs via the intermediate veins, with solute passing from mesophyll cells, via the mestome sheath cells to the phloem parenchyma or companion cells and finally to the sieve elements (Kuo et al., 1974; Figure 1.6).

Phloem loading is an active and selective process involving apoplastic transfer (there is no evidence which shows that plasmodesmata can discriminate between sugars or different forms of ions etc). A proton-co-transport system has been suggested for sucrose loading (Giaquinta, 1983; and Figure 1.7). But as Giaquinta

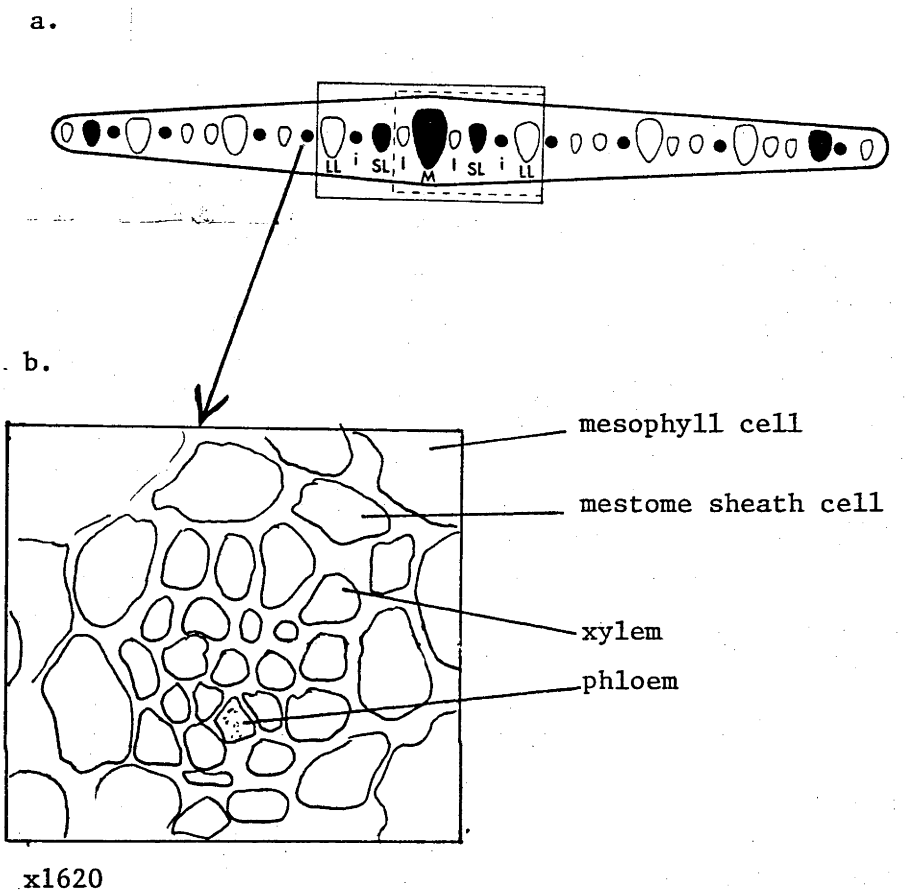
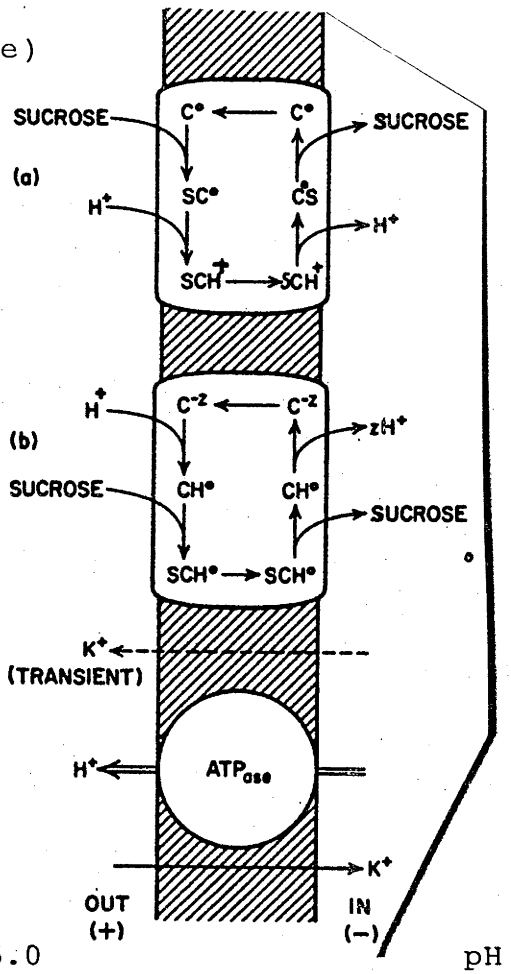


Figure 1.6 (a) Diagram of a transverse section of the third leaf blade of wheat (cv. Heron) about 15 cm from the base of the blade, showing vascular distribution. M midrib, LL large lateral, SL small lateral, L large intermediate, i small intermediate. The distance between two intermediates or a lateral and its adjacent intermediate average 250 μm (Altus and Canny, 1982).
(b) Tracing of cells which form the vascular bundle of a small intermediate.
- Kuo et al. (1974).

COMPANION CELL

SIEVE ELEMENT

(free space)



pH 5.5 - 6.0

low K^+

low sucrose

pH 7.5 - 7.5

high K^+ ((100 - 200mM)

high sucrose (0.8M)

Figure 1.7 Schematic model suggested by Giaquinta (1980a,b,1983) for sucrose loading across phloem membranes.
(a) C = uncharged carrier on the external membrane surface which binds with sucrose and proton(s)
(b) the carrier is shown as being negatively charged.
The manner in which these events are accomplished is not known.

(1983) points out, loading of different solutes may be via different pathways and the degree of apoplastic[1] and symplastic[2] transport may differ according to leaf morphology.

It would appear that the evolution of cereals has favoured the redistribution of phosphorus to the grain in preference to holding it in photosynthetic tissues. Marshall and Wardlaw (1973) found that the distribution of phosphorus from a leaf is largely controlled by the movement of carbon but in modern wheats there is a consistent negative relationship between grain yield per unit area and grain phosphorus concentration (Batten, unpublished). This suggests that further examination of the "link" between the translocation of carbon and phosphorus is warranted.

To increase yield per unit of phosphorus in the grain a higher C:P ratio in the phloem is required. This situation may occur in phosphorus deficient plants, provided the ATPase-generated proton gradient involved in translocation (Figure 1.7) is not suppressed by low ATP levels. This seems unlikely if only 0.3% of the total ATP derived from photosynthate is needed to meet the ATP requirement for active loading at the phloem as suggested by Sovonick et al. (1974).

The phloem parenchyma cells may be important for temporary storage of assimilates - a process involving either a symplastic or apoplastic pathway (Giaquinta, 1983).

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[1] cell to cell; through "free space"

[2] through plasmodesmata; along a downhill solute concentration gradient.

Transport

Phosphorus moves rapidly in the phloem (Koontz and Biddulph, 1957; Greenway and Gunn, 1966). In wheat, Marshall and Wardlaw (1973) calculated the speed of movement of ^{32}P and ^{14}C in the phloem at 68 and 69 cm hr^{-1} in the flag leaf sheath and 55 and 60 cm hr^{-1} respectively in the stem. These rates are similar to those reported for movement in the xylem from roots to ears of wheat (Frazier et al., 1956).

Carbon is transported predominantly as sucrose in wheat and phosphorus as inorganic ions. But much of the phosphorus in phloem exudate is organic sugar phosphates, uridine, adenine and guanosine nucleotide phosphates, phospholipids, and phosphorylethanolamine (Pate, 1976). Phosphorus is thought to have a buffering effect on the phloem contents and possibly controls the mobility of such cations as Ca^{++}, Ba^{++} and Pb^{++} due to the low concentration of free ions of these phosphate salts (Ziegler, 1975).

Various phosphatase enzymes have been localized in mature and differentiating phloem cells. Nucleoside phosphatase occurs in most sieve elements. The major sites of acid phosphatase include vacuoles and the endoplasmic reticulum of mature sieve elements and, in some species, the dispersed P-proteins of mature sieve elements. Both types of phosphatases occurs in companion cells and parenchyma cells (Cronshaw, 1980).

The high concentrations of ATP in the phloem (0.3 to 1.5 mM) and the rapid exchange with inorganic phosphorus suggest that this high energy phosphorus compound is involved in the mechanism of translocation and is a mobile form of energy (Bieleski, 1973; Pate, 1976). This is a complex and difficult area to research but a better

understanding of the role of phosphorus could be important for improving phosphorus utilization efficiency.

1.3.5 Starch deposition in grain in relation to phosphorus

Where the rachilla meets the pericarp of the grain the tracheary elements of the xylem are interrupted by a junction of transfer cells (Zee and O'Brien, 1970a). Solutes pass into the grain via the vascular bundle which extends along the ventral side of the grain and are apparently distributed through the chalazal zone (pigment strand) into the endosperm (Frazier and Appalanaidu, 1965; Figure 1.8). The cells on the endosperm side of the vascular bundle have thin walls traversed by many plasmodesmata. Duffus and Cochrane (1982) suggested that assimilate moves out of the symplast and into the apoplast in the transfer cells of the nucellar projection. But ... "how the assimilate moves into and through the endosperm is a matter of conjecture". Both symplastic and apoplastic movement have been suggested. Jenner (1980) favoured apoplastic transfer while Felker and Shannon (1980) noted plasmodesmata between endosperm cells.

Sucrose, the main carbon source for polysaccharide synthesis in the wheat grain (Duffus and Cochrane, 1982), apparently enters the grain from the phloem without hydrolysis (Jenner, 1976; Rijven and Gifford, 1983a).

The rate of enzyme-catalysed reactions (Duffus, 1979) and sucrose transport to and through the developing grain (Jenner, 1980), even the elastic properties of the grain outer layers (Duffus and Cochrane, 1982) may control the rate of starch deposition.

That phosphorus is essential for the formation of starch is well established, but some aspects are not agreed upon.

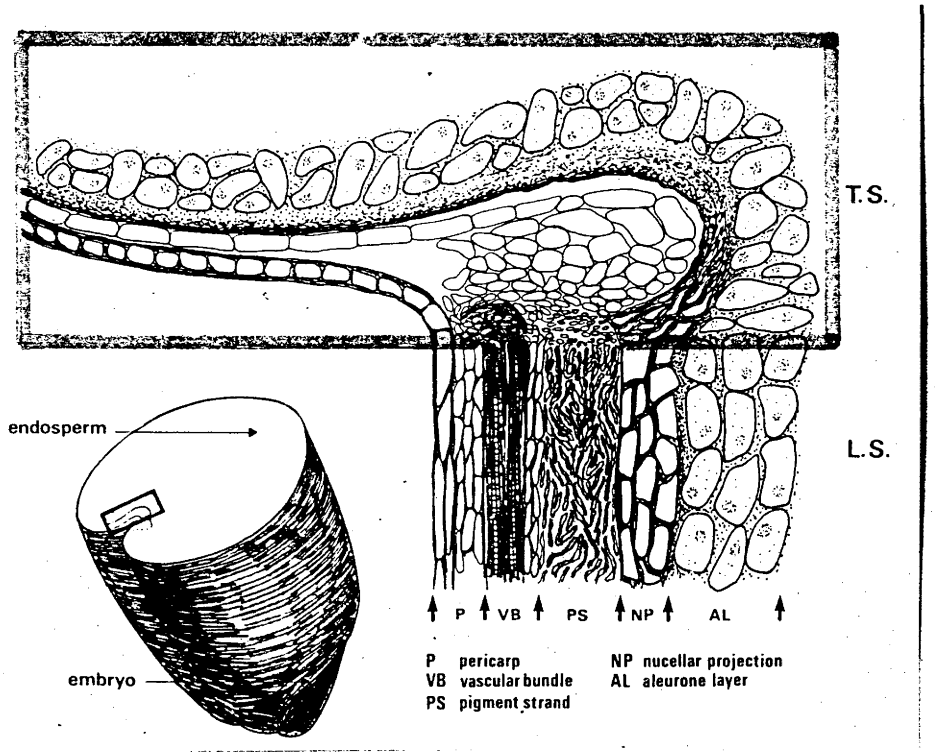

Figure 1.8 (a) Diagram showing half of a mature grain drawn in perspective. The area marked by the rectangle at the crease region is shown in detail in (b) A detailed view of the crease region. The upper portion of the figure represents a transverse view, while the bottom half shows a longitudinal view.
- Zee et al. (1970b).

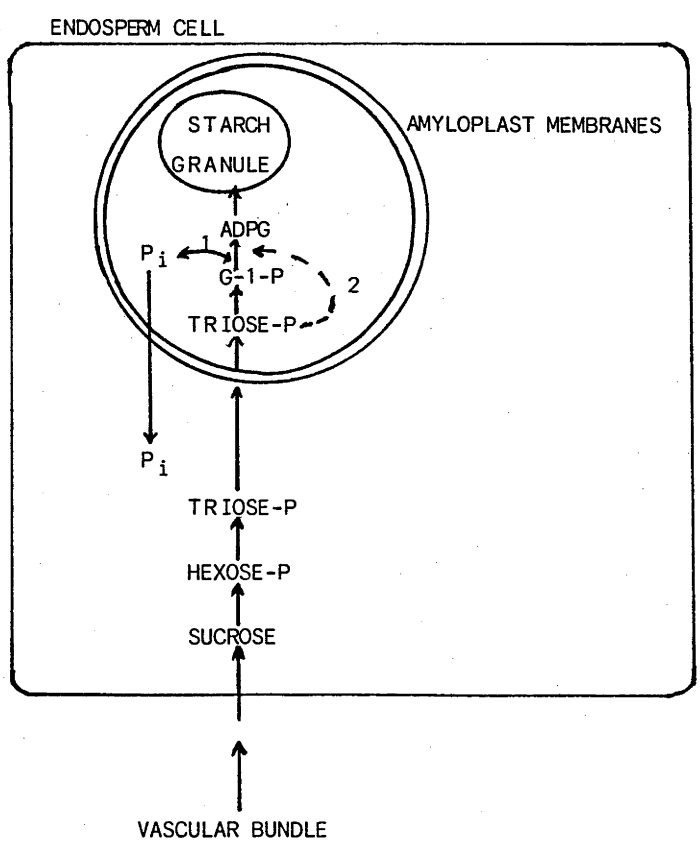

Figure 1.9 Scheme proposed by Jenner (1976) for the entry of assimilate into endosperm cells and amyloplasts (1) indicates feedback; (2) stimulatory process.

Jenner (1976) proposed that sucrose is degraded to triose phosphate in the cytosol of endosperm cells and this enters the amyloplast in exchange for inorganic phosphorus via a mechanism (Figure 1.9) similar to that reported at the chloroplast membrane (see section 1.3.2 above). This hypothesis has been supported by the study of Liu and Shannon (1981) on maize endosperm. These workers suggested that high P_i:G-1-P in the starch granule would favour starch breakdown, not synthesis.

This scheme does not appear to be supported by the findings of Rijven and Gifford (1983b). They found no obvious change in starch formation in endosperm slices when the inorganic phosphorus level was raised, by adding phosphorus, or lowered, by removing phosphorus with the sequestering agents mannose or myo-inositol. They did note a 15-18% decrease in ADPG pyrophosphorylase mediated starch synthesis in the presence of phosphorus at 25 mol m^{-3}.

A general scheme for the conversion of sucrose to a-(1,4)glucan (starch) in cereals presented by Duffus and Cochrane (1982) is shown in Figure 1.10. The major path to starch is via ADP-glucose synthesis (Preiss, 1982; Duffus and Cochrane, 1982).

The products of sucrose metabolism include UDP- and ADP-glucose, fructose and glucose. Duffus (1979) concluded that UDP-glucose is the major product and this is converted to starch via glucose-1-phosphate and ADP-glucose by the actions of UDP and ADP glucose pyrophosphorylase and ADP-glucose starch synthase. Some UDP-glucose may be converted directly to starch. Glucose and fructose formed during sucrose metabolism may be converted to glucose-6-phosphate and hence to starch. Starch synthase is most active with ADP-glucose.

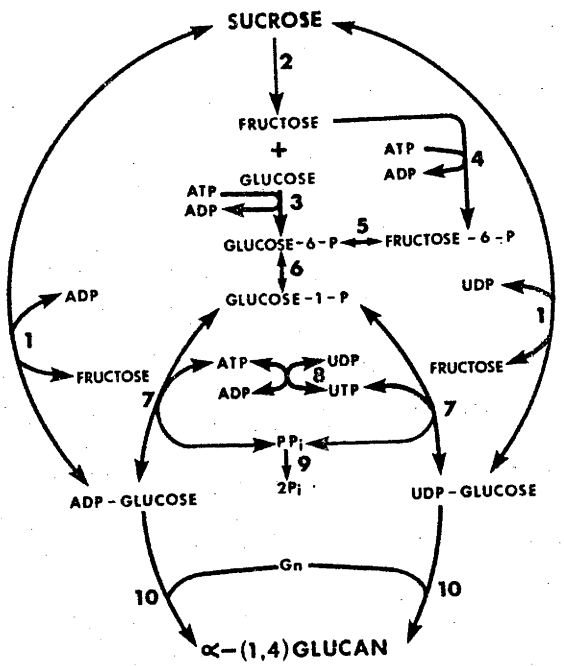

Figure 1.10 Some enzyme-catalysed reactions that could be involved in the conversion of sucrose to (1-4)-glucan in developing cereal endosperms, 1,UDP(ADP)-dependent sucrose synthase ; 2,invertase; 3, hexakinase; 4, fructokinase; 5, glucose phosphate isomerase; 6, phosphoglucomutase; 7, ADP(UDP)-glucose pyrophosphorylase; 8, nucleoside-diphosphate kinase; 9, inorganic pyrophosphatase; 10, ADP(UDP)- glucose starch synthase.
- From Duffus and Cochrane (1982).

Grain development represents a net synthesis and deposition of starch. There is evidence for the presence of both glycolytic and pentose phosphate pathway enzyme activity in developing barley endosperm and other reports of starch-degrading enzymes (Duffus and Cochrane, 1982). These aspects of starch metabolism in wheat are relevant to premature sprouting and the effects of high α-amylase activities on breadmaking quality.

Gordon (1980) and King (1983) reviewed sprouting in wheat. Grain phosphorus concentration has not been implicated in sprouting or dormancy problems in wheat but Jain et al. (1982) and Quick et al. (1982/83) have noted negative correlations between inorganic phosphorus and the depth of dormancy in oats.

1.3.6 Phosphorus deposition in grain

Whole, mature wheat grains have a total phosphorus concentration of between 0.08% (G.J. Blair, personal communication) and 0.76% (Sofield et al., 1977b).

The majority of the phosphorus in mature cereal grain is in the form of phytate (Figure 1.11, 1.12). Phytate received attention during World War II when it was shown that brown bread was inferior to white bread as a source of calcium in humans (McCance and Widdowson, 1942). Recently many nutritionists have singled out phytic acid and to a lesser extent fibre as the cause of low bioavailability of minerals from plant foods, even after allowance for the lower overall digestibility of plant food compared to animal products (Cheryan, 1980).

Even some American diets are deficient or marginal in ions such as iron, zinc, calcium and magnesium and the National Academy of

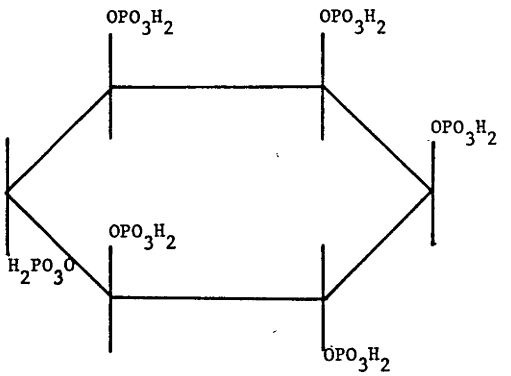

Figure 1.11 Phytic acid or myoinositol hexakisphosphate, the salts of which are referred to as phytate (Cosgrove, 1980a). The structure proposed by Anderson (1914) is shown here.

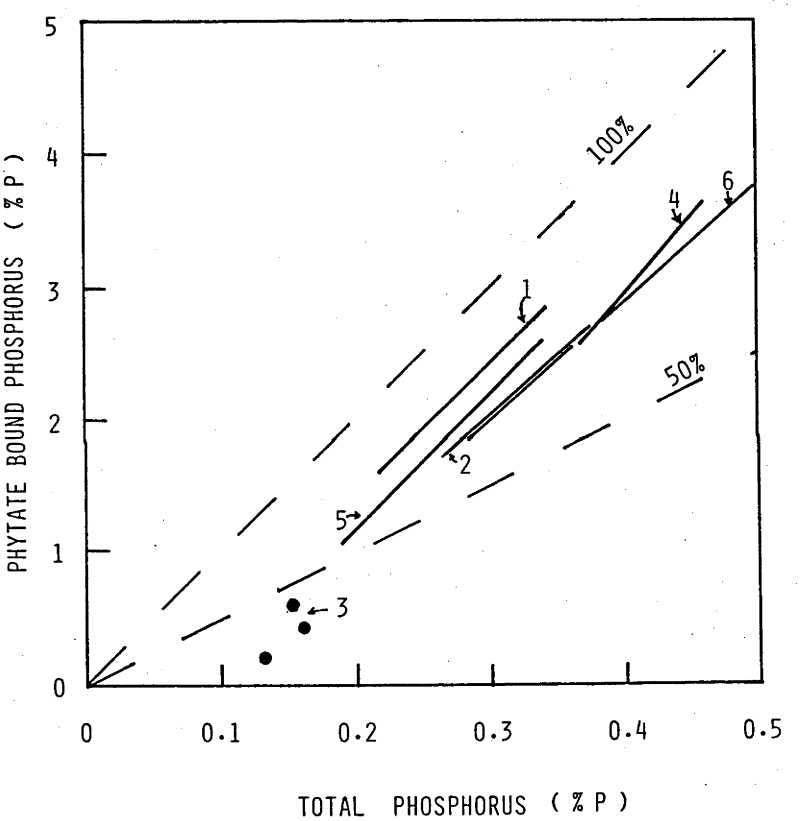

Figure 1.12 Examples of the relationship between total phosphorus (TP) and phytate bound phosphorus (PP) in wheat. The theoretical 50 and 100% relationship lines have been included to emphasize that PP%TP increases with TP and is usually > 50%.
Lines 1, 2 and 3 for wheat grown under dryland conditions at Wagga Wagga (Batten and Khan, unpublished data), 4 and 5 wheat grown in Iran (Nahapetain and Bassiri, 1976); 6 wheat grown in Michigan and Kansas (Lolas et al., 1976).

Science has proposed that cereal foods be fortified with these cations (NAS/NRC, 1974).

Nutrient deficiencies due to phytate are more evident in societies which consume wholemeal, unleavened grains. Factors affecting the destruction of phytate during breadmaking and the bioavailability of ions are not well understood (Tangkongchitr et al., 1981,1982; Erdman, 1981; Ranhotra et al., 1981). Thus a better understanding of factors affecting the formation of phytate in grains is required.

Phytate is deposited in discrete regions (called globoids) of cellular organelles which are usually referred to as protein bodies or aleurone particles (see Loewus and Loewus, 1983 for references; also Tanaka et al., 1974a,b; Ogawa et al., 1977). The formation and subsequent breakdown of phytate appear to occur only in these subcellular regions, i.e. phytin-containing particles are immobile (Loewus and Loewus, 1983). The distribution of phytate within the seed varies between species. In oilseeds (e.g. soybeans) phytate occurs throughout the kernel (Martinez, 1977; Maga, 1982). Wada and Maeda (1980) divided gramineous seeds into two groups after examining the size and frequency of globoids in aleurone and scutellar tissues and the total phosphorus content of embryos and endosperms.

Group	Features	Species
I	A large proportion of P in the aleurone layer, but both aleurone and scutellum rich in P.	Barley, oat, orchard grass, rice, rye, wheat.
II	Both the amount and the concentration of P in the scutellum were greater than in the aleurone layer.	Millets (barnyard, common, finger, Italian and Pearl), maize, sorghum, Job's tear (Coix sp).

Biosynthesis of phytate

Loewus and Loewus (1983) proposed the relationships thought to be involved in phytate formation, accumulation and breakdown in seed-bearing plants (Figure 1.13). The products of photosynthesis lead to glucose-6-P, 1L-myo-inositol-1-P and then to phytate via the actions of the enzymes 1L-MI-1-P synthase and phosphoinositol kinase. Loewus and Loewus (1983) cited reports which suggest that MI-1-P and MI-2-P are precursors of phytate. They concluded that there is little evidence as to the first phosphorylated intermediate leading to phytate.

Loewus and Loewus (1983) reviewed the available information on the enzymes shown in Figure 1.13. Briefly, the activity of synthase is stimulated by some ions, e.g. ammonium, and potassium, and inhibited by inorganic and pyrophosphate, sodium and a range of phosphorylated sugars. The synthase is present in cytoplastic and chloroplastic fractions of plants. In beans and in rice grains phytase shows a peak of activity 14-16 days after flowering.

Cosgrove (1980a) reviewed the five physiological roles suggested for the phytate found in seeds -

(a) A store of phosphorus. Inorganic phosphorus is liberated during germination by enzymatic hydrolysis which is practically complete after six days. There are no studies which show clearly the role of the phosphorus released during early seedling growth, or how much of the phosphorus is essential for seedling metabolism prior to the uptake of phosphorus from the soil. On the other hand, Loewus and Loewus (1980, 1983) suggested that the core substance, myo-inositol, may be as essential as the phosphorus it sheds.

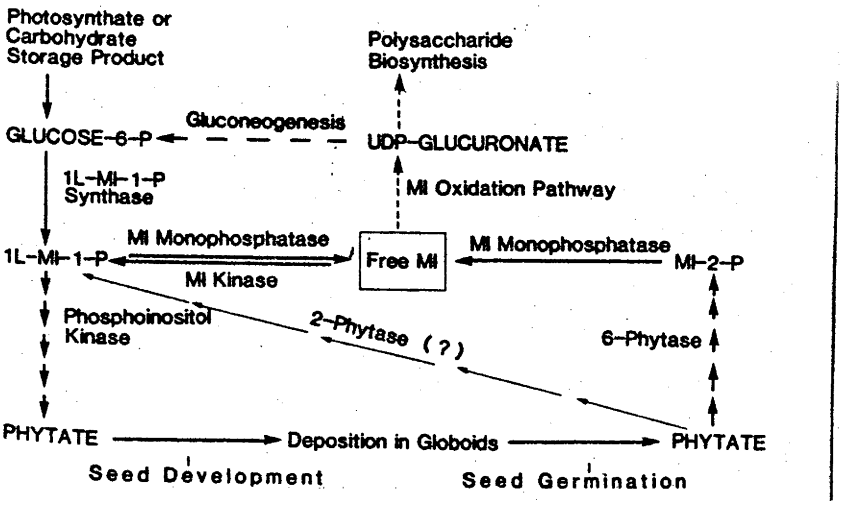

Figure 1.13 Schematic diagram of myo-inositol biosynthesis and metabolism as it is probably related to formation, accumulation and breakdown of phytate in seed-bearing plants.
Abbreviations: P,phosphorus; L, levo molecular configuration; MI, myo-inositol; UDP, uridine diphosphate glucose.
- Loewus and Loewus (1983).

Free inorganic phosphorus ions can inhibit hydrolysis of phytate (Bianchetta and Sartirana, 1967). This suggests that phytate phosphorus is not required for the germination and the establishment of wheat grown with phosphate fertilizer.

(b) An energy store. It has been suggested that charge repulsion between adjacent groups in the close-packed phytate molecule could result in relatively high phosphoryl transfer potential, and enhance protein synthesis in wheat endosperm. However, there appears to be no evidence to show that the stored energy value of phytate (which in wheat endosperm is insignificant) promotes germination.

(c) A competitor for ATP during its rapid biosynthesis near maturity, whereby metabolism is inhibited and dormancy induced. Again, this role has not been confirmed. A study in wheat by Williams (1970) showed that the ATP in developing grain does not fall to a low level until about seven days after the maximum rate of phytate formation.

(d) Chelation of multivalent cations. This may be associated with the slowing of metabolism and dormancy.

(e) Regulation of the inorganic phosphate level. In parenchyma cells the level of inorganic phosphorus is buffered at 5-20 mM by being isolated behind the membrane of the vacuole. In the developing seed the vacuole disappears and phytate may assume the role of a buffering agent for phosphorus (Bieleski, 1973; Heldt et al., 1977; Michael et al., 1980).

The proportion of phosphorus as phytate increases with the total phosphorus concentration. If phytate is simply a storage form of phosphorus then it should be possible to produce zero-phytate grain by growing plants with the "critical" phosphorus concentration. Extrapolation of linear relationships in Figure 1.12 indicate that this may be achieved in plants which produce grain with a total phosphorus concentration of 0.1%.

Wheat grown in a very dry season (1977) at Wagga Wagga (3 in Figure 1.12) was found to contain only 0.13% phosphorus with only about 13% of this bound as phytate. However, the phytate bound phosphorus was determined using an indirect iron precipitation technique (Wheeler and Ferrel, 1971). These data and those of several other workers may be misleading. Lower esters of inositol precipitate ferric iron in acid solutions, the ratio of iron to phosphorus in the precipitate has been reported to vary from 2.46–4:39:6 (Wheeler and Ferrel, 1971; Thompson and Erdman, 1982), the presence of ferric iron in the plant material itself, and the precipitation ratio of Fe:P is not the same in hydrochloric and sulphuric acid solutions (Cosgrove, 1980a). If the precipitate is analysed for phosphorus directly then errors may be due to inorganic phosphorus which has co-precipitated (Ellis et al., 1977).

Recently, an ion-exchange resin technique has been reported by Cosgrove (1980b). This was adopted (Chapter 2) for the studies reported in this thesis.

1.4 SUMMARY AND HYPOTHESIS

This review highlights the essential role of phosphorus in the major aspects of grain filling. An important question is "why is phosphorus retranslocated along with assimilate during grain filling and how is this association regulated?" When phosphorus is translocated from vegetative tissue photosynthesis may decline and the tissue may eventually senesce, and thereafter contribute nothing towards grain dry weight accumulation. Much of the phosphorus deposited in the grain is apparently stored as phytate, a compound which is undesirable for human nutrition.

This thesis examines the hypothesis that modern wheats use phosphorus inefficiently during grain development. Experiments are reported which show the consequences to photosynthetic tissue and the lack of response by grains to the translocation of phosphorus in plants grown with a limited supply of phosphorus.

CHAPTER 2:

MATERIALS AND METHODS

2.1 INTRODUCTION

2.2 THE WHEAT CULTIVAR

2.3 PLANT CULTURE

2.3.1 Glasshouse

2.3.2 Root medium and nutrients

2.3.3 Plant protection

2.4 REPLICATION AND EXPERIMENTAL LAYOUT

2.5 ASSESSMENT OF PLANTS

2.5.1 Height

2.5.2 Leaf area

2.5.3 Anthesis

2.5.4 Maturity

2.5.5 Photosynthesis

2.6 DRYING AND GRINDING SAMPLES

2.6.1 Oven drying

2.6.2 Freeze drying

2.6.3 Grinding

2.7 CHEMICAL ANALYSES

2.7.1 Total phosphorus and nitrogen

2.1 INTRODUCTION

Fluxes in soil water, other nutrients, temperature and other environmental variables interact to confound effects caused by phosphorus. For this reason all the experiments reported here were conducted in the controlled environmental conditions of the Canberra Phytotron. The materials and methods described in this chapter refer to experiments reported in Chapters 3-8.

2.2 THE WHEAT CULTIVAR

The wheat used in most experiments reported here was Triticum aestivum L. (cultivar Kite). Kite is a potentially high yielding, short statured bread wheat which carries the Gai/Rht 2 dwarfing gene from Norin 10-Brevor (Gale et al., 1981) and the Sr26 gene for stem rust resistance from Agropyron elongatum (Ferns et al., 1975). The parentage of Kite is shown in Figure 2.1. Kite was separated out by hierarchical classification analysis of phenotypic relations as a cultivar with a long ear and heavy chaff (Syme and Thompson, 1981).

Kite was selected as a suitable wheat cultivar for these studies because it is potentially high yielding and in 1983 was a "recommended variety" for sowing in four of the six Silo Groups in New South Wales (Cook, 1982). As a plant for use in pot experiments Kite was considered suitable because of its short stature, negligible vernalization or photoperiod responses (Dr. J.L. Davidson, personal communication), and absence of awns except at the tip. One slight disadvantage is that, the flag fleaf tends to curl. Gas exchange measurements in some leaf chambers or porometers could be more difficult on these compared to flat leaves.

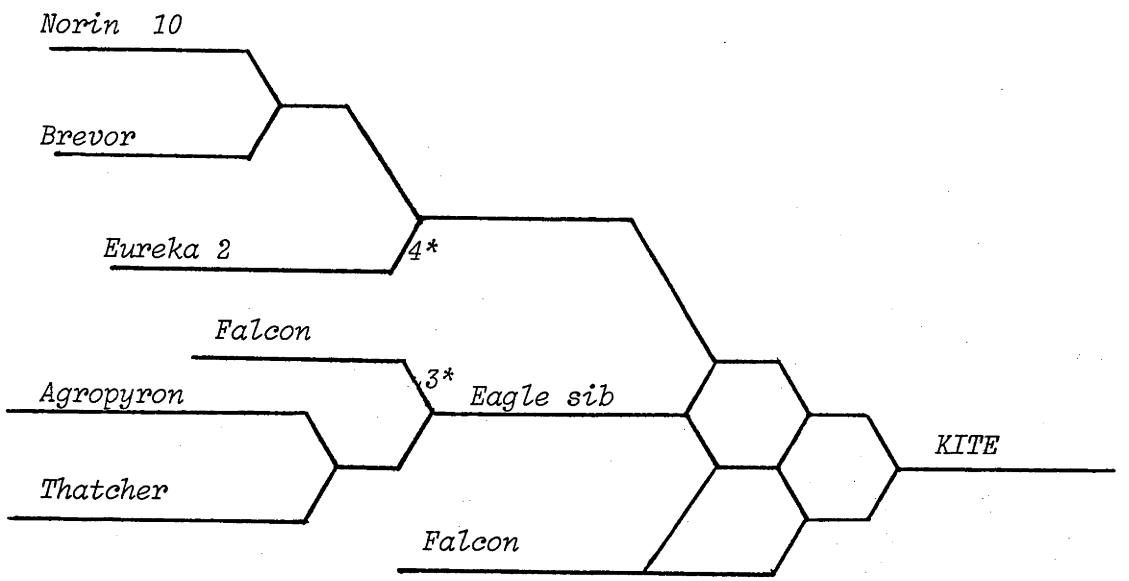

Figure 2.1. Parentage of Triticum aestivum L (cv. Kite). 3* and 4* indicate the number of times a parent was used in a backcross - from Moss and Wrigley (1974).

2.3 PLANT CULTURE

2.3.1 Glasshouse

The plants were grown in open glasshouses of the Canberra Phytotron. The temperature regimes varied according to the experiment but always with a 5°C difference between the day temperature (8 hours) and the night temperature (16 hours). The natural daylight was extended to 16 hours with low intensity (10 μmole m^{-2} s^{-1} at plant height) incandescent lamps.

2.3.2 Root medium and nutrients

Single plants were established in 127 mm plastic pots which contained 1 kg of dry river sand. To obtain a uniform population of plants, grains were taken from plants grown in a glasshouse and germinated prior to sowing.

Nutrients were supplied to the plants each morning in a modified Hoagland's No. 2 solution which contained 1 mM, 0.25 mM or nil inorganic phosphorus. The 0.25 and nil P solutions were used to produce plant with low phosphorus concentrations (hence the term low P). Preliminary experiments to show the effects on plants of modifying the concentration of phosphorus in the nutrient solution are described in Chapter 3. The likely effects of low phosphorus and of modifying the ammonium:nitrate nitrogen ratio in the nutrient solution are discussed in Appendix 1.

The plants were watered with tap water each afternoon.

River sand was used in these studies to facilitate the extraction of roots and to minimise retention of phosphorus in the root zone in Low P treatments. Prior to use in the experiments the sand held 2 $\mu g\ g^{-1}$ of bicarbonate extractable phosphorus. This increased when nutrient solution was applied (Appendix 2). The pH of the sand remained above 6.0 regardless of the phosphorus regime.

The roots of Kite and other wheat grown in this sand were found to be free of pathogenic and mycorrhizal fungi. This was to be expected following the methyl bromide sterilization of all material used in the Phytotron.

2.3.3 Plant protection

Red spider, aphids and powdery mildew were controlled by the sprays Omite®[1], Pirimer®[1] or DDT, and Bayletor®[1] respectively. In later experiments effective biological control of red spider was achieved by a cultured population of the predatory mite Phytoseiulus persimilis (Athias-Henriot) and Typhlodromus occidentalis (Nesbitt).

2.4 REPLICATION AND EXPERIMENTAL LAYOUT

A preliminary experiment was conducted to establish the number of replicates required to obtain standard errors of the treatment means which would be significant at the 5% level of significance. The main findings are presented as Appendix 3.

Plants were placed on trolleys in groups of 16 or 42 per trolley (64 plants m^{-2}). All the plants on a trolley received the same nutrient solution. The positions of the plants on a trolley and trolleys in the glasshouse were moved regularly to reduce positional effects such as variation in light intensity, temperature, air movement, humidity, CO_2 level etc.

2.5 ASSESSMENT OF PLANTS

2.5.1 Plant height was the distance from the surface of the sand in the pot to the base of the ear.

2.5.2 Leaf area was determined using an electronic planimeter (Li.COR, model Li-300; Lambda Instruments Corporation, Lincoln, Nebraska).

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[1] Specific data on the potential toxicity of chemicals used in these studies are included as Appendix 4.

2.5.3 Anthesis was recorded as the time of extrusion of anthers from the glumes of central ear spikelets. Upper and lower ear spikelets reached anthesis several days later.

2.5.4 Maturity, or the completion of grain filling, was visually assessed by the loss of chlorophyll from the glumes and grains. (See Chapter 4 for comparison of techniques).

2.5.5 Photosynthesis, here considered as the net carbon exchange or N.C.E., was measured in an open ended cuvette system. One or two leaves were placed in a perspex, water cooled chamber with an internal cross section of 5 x 3 cm. Air, drawn from above the building was mixed in a large container and passed through the leaf chamber. The flow rate was varied so that N.C.E. was independent of the flow rate. This method was considered to remove the effect of variable boundary layer resistance which results from leaf area and N.C.E. rate differences. The leaf chamber was illuminated by the bank of fluorescent tubes of an L.B.H. cabinet (Morse and Evans, 1962) plus a 1000 W metal-halide lamp (HPLR, Philips, Holland). The photon flux density of radiation in the 400-700 nm wavelength range on the upper surface of the leaf was 1160 μmoles m^{-2} s^{-1}. The leaf temperature was measured using a copper-constantan thermocouple junction pressed against the abaxial (lower) surface of the leaf. Leaf temperature was maintained at 1°C above the ambient temperature in the glasshouse by means of the water jacket cooling system.

The carbon dioxide in the air before and after passing through the leaf chamber was measured using an A.D.C. series 225-2B-SS

infra red gas analyser with interference filters to eliminate cross-sensitivity to water vapour. In addition both streams of air entering the IRGA were dried over calcium chloride. The N.C.E. was calculated from the differential in CO_2 and the flow rate.

2.6 DRYING AND GRINDING OF SAMPLES

2.6.1 Oven drying

Samples required for dry weight, total phosphorus and nitrogen and gross radioisotope analyses were dried to constant weight at 65°C in a laboratory oven with inbuilt fan.

This temperature removes about 98% of the moisture relative to drying at 105°C but minimises losses (especially of nitrogenous compounds) due to thermal decomposition (Jones and Steyn, 1973).

2.6.2 Freeze drying

Fresh samples required to determine phosphorus fractions (soluble, lipid, phytate or residual forms) were frozen in liquid nitrogen (-196°C) then lyophyl ized (-30°C at 100 μ mercury) to constant weight and stored at -25°C.

2.6.3 Grinding

Samples were ground (or crushed) to smaller particles when a homogeneous sample was needed for analysis or where complete extraction of a compound depended on exposing a large surface area to an extracting solution. For vegetative material, where subsamples as small as 20 mg dry matter were taken, samples were passed through a Wiley Mill fitted with a 40 mesh screen. Grain samples (of two or more grains) were more efficiently powdered by crushing for 10 to 30 seconds in a chromium alloy ball mill agitating at 1000

movements min^{-1} (N.V. Tema, Labor Scheibenschwingmuhle, t250 fitted with a 52 mm diameter 500 g weight within a 68 mm diameter ring).

2.7 CHEMICAL ANALYSES

2.7.1 Total phosphorus and nitrogen

Dry ground plant material (20 to 100 mg) was digested in test tubes at 330°C by the Kjeldahl method (Williams and Twine 1967), using dry matter and digestion mixture in the ratio of 0.1 g to 5 ml. The digestion mixture of sulphuric acid (1 L) with potassium sulphate (100 g) to increase the boiling point, and selenium (1 g) which is a catalyst, was prepared by boiling in a round bottomed flask fitted with a reflux condenser.

The digests were diluted to 20 times the volume of digestion acid used and the total orthophosphate and ammonium nitrogen were determined colorimetrically using an autoanalyser. For phosphorus the molybdenum blue method was used with ascorbic acid as the reducing agent. For ammonium the blue nitroprusside complex method was used (Technicon, 1977).

Grain samples supplied by the Bread Research Institute, Ryde N.S.W., were used as check standards.

2.7.2 Fractionation of phosphorus in vegetative tissues.

Total phosphorus was separated as shown in Figure 2.2 into soluble, ester, lipid and residue (DNA, RNA) fractions using a procedure based on Chapin and Bieleski (1982). Freeze dried, ground plant material was extracted with methanol:chloroform:formic acid:water (MCFW; 12:5:1:2 v/v) overnight at -25°C, centrifuged,

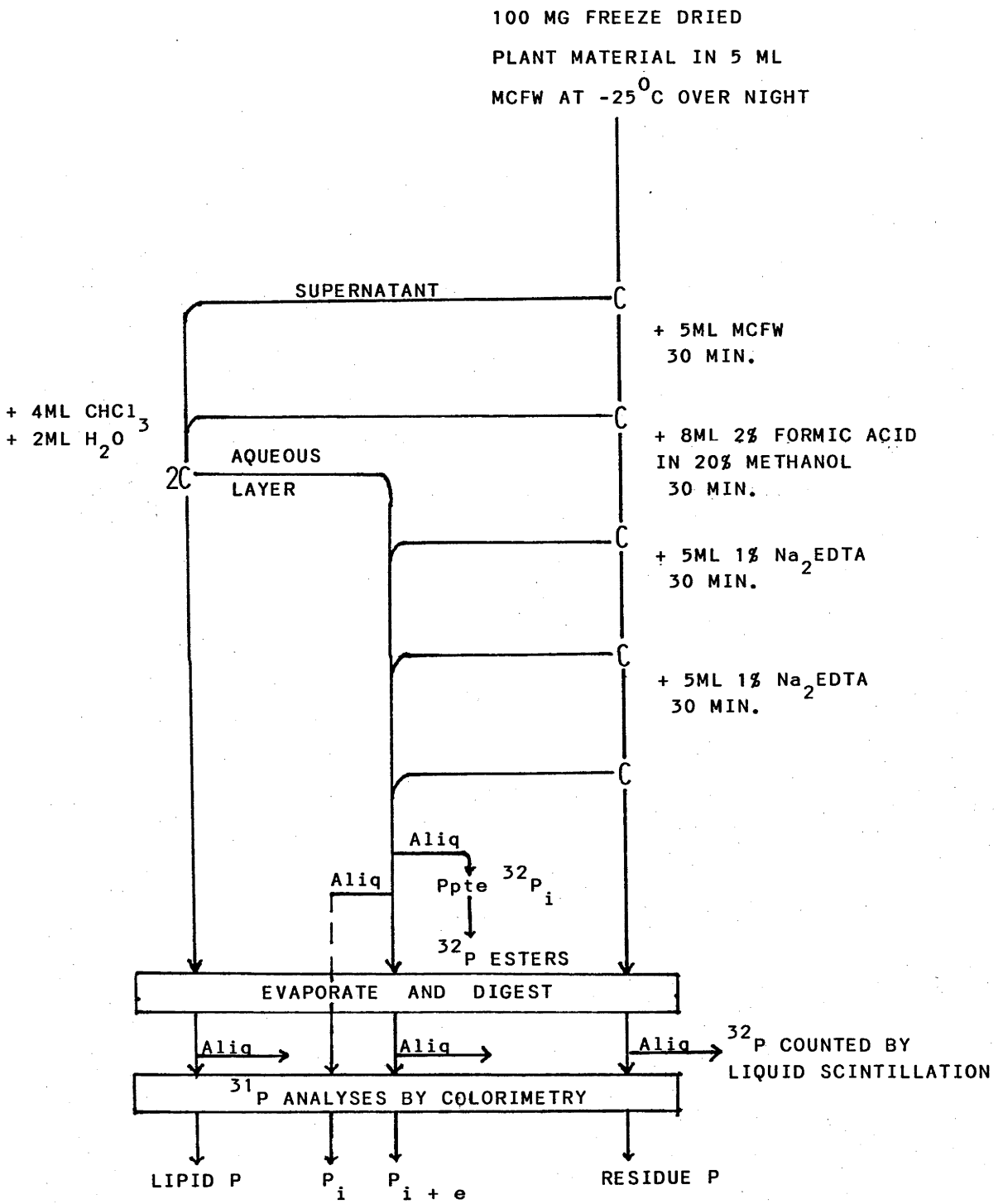


Figure 2.2. Flow diagram for fractionating phosphorus in vegetative tissues
MCFW = methanol, chloroform, formic acid, water (12:5:1:2)
C = centrifugation at 3000 g for 10 min (C) or 20 min (2C);
Aliq = aliquot; P_i = inorganic and P_e = ester phosphorus
- after Chapin and Bieleski (1982).

decanted, re-extracted and centrifuged to ensure more complete separation of soluble and residue compounds. The combined supernatants separated into two layers following additions of chloroform and water and adequate centrifuging. There was very little white curd (RNA with less than 1% of total phosphorus, Chapin and Bieleski, 1982) and to reduce contamination of the lower chloroform (lipid) layer this curd was removed with a pasteur pipette and included in the upper aqueous fraction (inorganic and ester compounds).

The residue was successively washed with methanol-formic acid and twice with Na_2EDTA to remove chelated inorganic phosphorus. The combined aqueous supernatants were adjusted to known volume. An aliquot was taken for direct analysis of inorganic phosphorus (^{31}P) or for analysis of ester phosphorus (^{32}P) after precipitating orthophosphorus with triethylamine hydrochloride (Sugino and Miyoshi, 1964).

The total phosphorus in each fraction was obtained after evaporating the extracts, or residue, to dryness in a water bath under an air stream and digesting by the Kjeldahl method above. An aliquot of the Kjeldahl digest was take for ^{32}P analysis.

2.7.3 Fractionation of phosphorus in grain

2.7.3.1 Extraction and fractionation

The total phosphorus in grains was separated into trichloroacetic acid (TCA) soluble and insoluble forms. Phytate phosphorus was separated from the former using the anion exchange resin Dowex AG1-x2 200-400 mesh (Cosgrove 1980b; Figure 2.3).

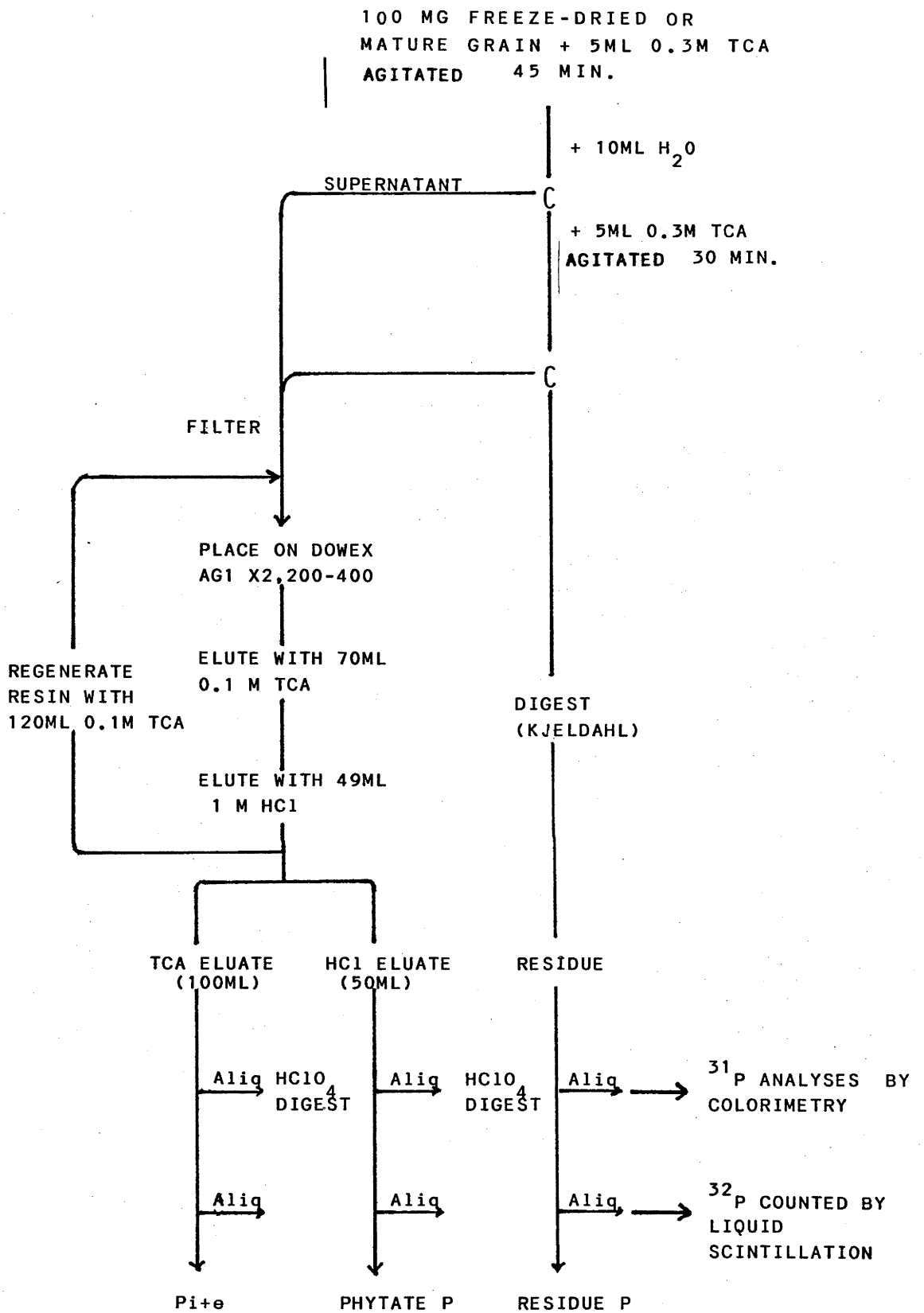


Figure 2.3. Flow diagram for fractionating phosphorus in grain
TCA = trichloroacetic acid
C = centrifugation at 3000 g for 10 min
aliq = aliquot
i + e = inorganic plus ester phosphorus.

Freeze dried grain (100 mg) was extracted twice with 5 ml 0.3 M trichloroacetic acid (TCA). ^{32}P analyses showed that the second extraction removed 6% of the total acid extractable phosphorus in the combined extracts. The extracts were diluted with 2 parts of water centrifuged, filtered and placed on the resin column. Additional 0.1 M TCA was used to elute inorganic and inositol-1-phosphate. Phytate bound phosphorus was eluted using 1 M HC1.

Complete recovery of the phosphorus added to the columns was achieved. After elution with HCl the columns were flushed with water to remove free chloride. They were subsequently recharged with 20 bed volumes of 0.1 M TCA just before reuse.

2.7.3.2 Determination of phosphorus

An aliquot (1 ml) of each eluate was digested with 0.2 ml of 70% perchloric acid at 180°C for 1½ hours. The total phosphorus in the aliquot was determined using the molybdenum blue method. For the initial samples stannous chloride was used as the reducing agent (Dickman and Bray, 1940). This was later discarded in favour of the more sensitive and more convenient ascorbic acid method of John (1970). The grain residue fraction was digested by the Kjeldahl method (2.7.1) analysed after neutralization with NaOH using p-nitrophenol as the indicator.

2.7.3.3 Phosphoric esters in immature grain.

Cosgrove (1980b) found no intermediate esters of inositol in mature peas but suggested that other samples should be checked before conducting routine analyses.

Two samples of immature grain from Experiment 2 (taken 22 days after anthesis) were subjected to gradient elution with 0 to 1 N HCl

after the method of Parr (1954) using the apparatus of Cosgrove (1980b). Each fraction collected had 430 drops (about 23 ml/fraction). From each fraction 1.0 ml was digested and analysed for phosphorus. Selected fractions were titrated against NaOH to obtain the acidity of the eluate.

2.7.4 Chlorophyll

Fresh leaf discs (10 mm diameter) or discs stored at -25°C in the dark were ground using a pestle and mortar in 85% acetone (10 ml) with calcium sulphate as an abrasive agent. The extract was centrifuged and the absorbance of light at the wavelengths 645 and 662 nm recorded in 10 mm curvettes. Chlorophyll a and b were calculated according to Arnon (1949).

2.8 RADIOACTIVE ISOTOPES

2.8.1 Phosphorus (^{32}P) labelling

$H_3{}^{32}PO_4$ in HCl supplied by the Australian Atomic Energy Commission was diluted with 1,3-bis[tris(hydroxymethyl)methylamino] propane (BIS-TRIS Propane) buffer at pH 6.5 to give an activity of 74×10^3 or 185×10^3 Bq 10 ml^{-1}). The pH of the buffer was not changed by mixing 2:1 with the ^{32}P-HCl solution. For labelling 10 µl of buffer-^{32}P solution, was applied to the adaxial surface of the flag leaf with a fine brush with up to 90 µl of water containing 0.1% Tween 20 solution.

2.8.2 Carbon (^{14}C) labelling

The flag leaf blade was placed across a leaf chamber, similar to that described in section 2.5.5, with a photon flux density at the leaf of 500 µmole m^{-2} s^{-1} and the cabinet temperature the same as the day

temperature used to grow the plants. To label the plant air containing generated $^{14}CO_2$ was circulated through the leaf chamber at 3.8 L min^{-1} for 5 minutes. The $^{14}CO_2$ was generated by adding excess warm 50% lactic acid to 50 mg of Ba $^{14}CO_3$ with an activity of 9.25×10^7 Bq mM^{-1}. Excess ^{14}C was then removed using a by-pass through soda lime (Figure 2.4).

2.8.3 Post labelling conditions

Immediately after being labelled plants were kept in an artificially lit L.B.H. cabinet (Morse and Evans, 1962). The temperatures were similar to those in the glasshouse and the photon flux density at the level of the flag leaves was 530 µmoles m^{-2} s^{-1}. The relative humidity was maintained at 75%.

2.8.4 Counting radioactivity and calculation of redistribution

2.8.4.1 Dry samples

Dry ground tissue (mg) was placed in stainless steel planchets and compressed with a hand-held plunger to give a sample of uniform depth and uniform distance from the detector. The radioactivity was counted with a low background, helium-butane gas filled, thin end window Geiger-Müller detector (ICN, Tracerlab). Dual labelled samples were counted twice; once for total activity from both isotopes and then with a layer of plastic to shield the low energy ^{14}C radioactivity. The plastic was found to eliminate 99.7% of the ^{14}C counts and 25% of the ^{32}P counts. The activity of each tissue was expressed in relative terms, vis:

Relative activity for tissue = cpm for sample x dry weight of tissue

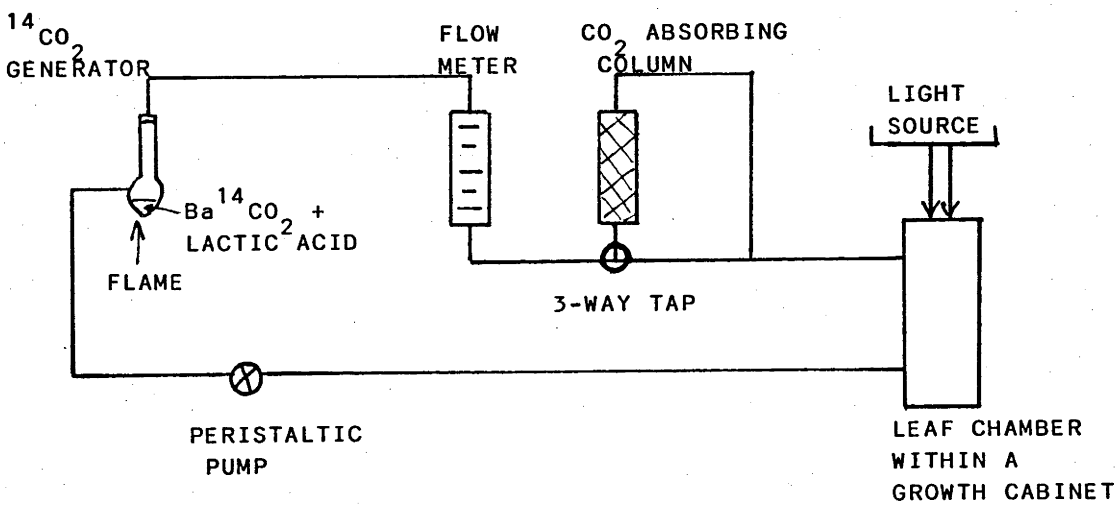


Figure 2.4. Diagram of $^{14}CO_2$ generator and leaf labelling system.

This relative activity was then calculated as a proportion of the total relative activity for the plant.

2.8.4.2 Tissue extracts

The ^{32}P radioactivity in solutions from the fractionation methods (2.7.2 and 3) was measured using the liquid scintillation technique. Aliquots (50 to 1000 ml) of extract were mixed vigorously in glass vials with 10 ml of scintillation fluid containing Toluene, Triton X-100 and PPO (2,5-diphenyloxazole) in the ratios 1334 ml:666 ml:8 g.

The vials were left in the dark until chemiluminescence subsided. If chemiluminiscence persisted for more than 24 hours 200 µl of 15% ascorbic acid was added to the vial. ^{32}P disintegrations were counted using a Beckman model LS6800 liquid scintillation counter.

The degree of quenching (of the light emitted by the scintillant in response to the ionizing particles) was checked for each radioactive solvent. Effects due to quenching were eliminated by varying the size of the aliquot (e.g. < 200 µl for sulphuric acid but 1000 µl for TCA extracts).

CHAPTER 3

GROWTH AND REDISTRIBUTION OF PHOSPHORUS IN WHEAT GROWN WITH DIFFERENT PHOSPHORUS AND TEMPERATURE REGIMES

3.1 INTRODUCTION

3.1.1 General aim

The experiments reported in this chapter were carried out so as to gain an understanding of the effects of various phosphorus regimes on the relation between growth and the uptake and distribution of phosphorus by the wheat plant. In one experiment growth up to anthesis was varied independently of the supply of P by varying air temperature.

3.1.2 Phosphorus regimes

It is widely believed that cereals such as wheat acquire enough phosphorus during the first four weeks of growth to produce maximum yields. This belief arises from several early studies carried out in culture medium at glasshouse temperatures. Gericke (1924) made the following statement:

> "It appears, therefore, that while phosphorus is needed in the early growth period of wheat, it is not only useless, but relatively harmful, if present in physiologically available form in the culture media for the latter growth period" .

Indeed, Gericke's statement was based on observations of large differences. The plants grown with phosphorus for only four weeks were 125 cm tall and produced 700 g of fresh matter while the plants supplied with phosphorus for 12 weeks were only 90 cm tall and weighted only 480 g. These differences may have been due to an imbalance between the major nutrients or deficiency of essential elements in the nutrients solution used to growth the plants.

Brenchley (1929) found that barley supplied with phosphorus for six weeks or longer produced maximum number of tillers, ears and

grain and more dry weight. Boatwright and Viets (1966) found that wheat supplied with 2 mM P solution changed weekly for the first 5 weeks produced the maximum dry matter and grain. Chapman and Keay (1971) showed that wheat plants grown in sand responded to phosphorus supplied up to ear emergence (2 mM P solution applied to the sand every third day).

Sutton et al. (1983) reported that a supply of phosphorus (1.4 mM P in aerated nutrient solution) from germination to noding was sufficient to produce maximum dry matter, while for maximum grain yield phosphorus needed to be supplied until the mealy ripe stage of grain development, however, reexamination of the data in that paper suggests that grain yield was maximal if phosphorus was only supplied to heading.

It appears that it is not the period for which phosphorus is provided that is critical to growth and grain development but the ontogeny of the plant. However in all of these studies only one level of phosphorus was applied (for varying lengths of time) and the tissue phosphorus concentrations were mostly higher than would be found in plants in the field. In Experiment 1 below plants were grown with both the standard nutrient solution supplied in the Canberra Phytotron (1 mM P) and with the same solution modified to 0.25 mM P. The latter solution, when applied for a short period, produced smaller (not stunted) plants with features akin to plants grown in the field (in Australia). By using two phosphorus levels it was possible to test for interaction effects between the level and the time for which phosphorus was applied.

3.1.3 Temperature regimes

Sutton (1969) stated that, temperature must affect the rate of release of phosphorus from the solid phase into the soil solution and the rate of diffusion and he quoted studies which reported that isotopically dilutable and anion exchange resin extractable phosphorus increase with temperature. He also noted that the equilibrium concentrations of phosphorus in the soil solution increase by 1-2% with each degree rise in temperature. In contrast Chien et al. (1982) concluded that sorption increased but desorption decreased with increasing temperature when both sorption and desorption were initially at the same temperature. This agreed with earlier work by Beaton and Read (1963) and Barrow (1974) and suggests that more fertilizer is required to maintain a given level of soil solution phosphorus in a warmer soil. But, like Singh and Jones (1977), Chien et al. (1982) found that when desorption followed sorption at 25°C the amounts of phosphorus desorbed increased with temperature. In this case less fertilizer would be required by plants grown at a higher temperature. Singh and Jones (1977) stated that physiological effects of temperature on lettuce plant growth and consequent phosphorus requirement played only a minor role relative to sorption.

The concentration of phosphorus in plant tissue represents a balance between dry matter accumulation and phosphorus uptake. Power et al. (1964) found that at low soil temperatures (7-11°C) the uptake of phosphorus continued at a relatively rapid rate and increased the tissue concentration. At higher temperatures (up to 27°C) dry matter production was limited by the uptake of phosphorus. On the other hand, Smika and Ellis (1971) found that plant phosphorus levels early in the growing season were not changed

when the soil temperature was varied from 3° to 10°C either rapidly or slowly.

The concentration of phosphorus in wheat grain is lower in plants grown at lower temperatures (Sofield et al. 1977b). This may be caused by a lower uptake rate per unit weight of root (Yoshida et al. 1978) or by a lower rate of transfer of phosphorus, relative to carbon, to the grain. The effects of temperature on plant phosphorus are likely to be much greater when plants are growing in phosphorus deficient media.

In Experiment 2 below, plants were grown with a low phosphorus regime and subjected to a range of pre-anthesis temperatures. This effectively changed the length of the period of growth prior to anthesis, the supply of phosphorus to the plant, and the size of the plant at anthesis. It is shown that the severity of the low phosphorus regime at both anthesis and during grain filling was modified by the pre-anthesis temperature.

3.2 MATERIALS AND METHODS

3.2.1 Experiment 1: Phosphorus regimes

Wheat (cv. Kite) plants were grown as described in Chapter 2 in a glasshouse controlled to 24°C during the day and 19°C at night.

Nutrient solutions which contained 1 mM, 0.25 mM, or nil mM P (Chapter 2.3.2), were used to produce eight phosphorus regimes, vis. 2 levels of phosphorus x 4 periods of application after sowing (Table 3.1). Five replicates (single plants) were harvested at anthesis and four at maturity.

3.2.2 Experiment 2: Temperature regimes

Wheat (cv. Kite) plants were grown, as described in Chapter 2. During the first 20 days the plants were in a glasshouse controlled to 24°C by day and 19°C at night and were given nutrient solution containing 0.25 mM P.

On day 20, the seedlings were at the spikelet differentiation stage or growth stage 6 according to the scale reported by Nerson et al. (1980). Between day 20 and anthesis the plants were held in glasshouses controlled to the following temperature regimes (°C)

Day (8 hours)	15	18	21	24	27	30
Night (16 hours)	10	13	16	19	22	25

From day 21 to maturity the plants were supplied with nutrient solution free of phosphorus. To obtain a uniform grain filling period plants were transferred at anthesis to a glasshouse controlled at 18°/13°C.

Plants were harvested from each temperature regime at anthesis and at maturity (of the main culm) and from two treatments during grain filling. At anthesis subsamples of the flag leaf were weighed fresh then stored at -25°C for subsequent analyses. Other tissues were oven dried (Chapter 2.6).

The mature main culm heads were dissected into upper, lower, and central spikelet grains. The central spikelet grains were further subdivided into A + B florets and C + D florets.

The net carbon exchange rate of the flag leaf blade was followed during grain filling by monitoring at least six plants per temperature regime (Chapter 2.5.5).

Total phosphorus, nitrogen, chlorophyll and grain phytate were measured as described in Chapter 2.7. Soluble phosphorus was extracted from frozen tissue using sulphuric acid (Bouma and Dowling, 1982).

3.3 RESULTS AND DISCUSSION

3.3.1 Experiment 1: Effects of phosphorus regimes

There were few significant interactions between phosphorus concentrations and the time of phosphorus application on plant attributes at anthesis or maturity.

The interaction observed in plant total dry weight (Table 3.2) was due to continued production of tillers where phosphorus was supplied at both 1 and 0.25 mM P during grain filling in contrast with senescence of some tillers where 0.25 mM P was only supplied to floral initiation (Table 3.3). The decline in shoot:root ratio (Table 3.3) in plants supplied with phosphorus during grain filling was possibly due to a depression of shoot or grain growth, and inclusion of crown material in the root component.

The non significant depression in grain yield where 1 mM P was supplied during grain filling (Table 3.3) is consistent with the results of Brenchley (1929), Chapman and Keay (1971) and Sutton et al. (1983).

The main culm became a smaller proportion of the total shoot and contributed less to grain yield as the phosphorus supply increased. In very deficient plants 60% of the shoot or grain was found in the main culm, compared to < 25% in plants supplied with 1 mM P to flag leaf emergence or longer (Table 3.4).

The grain filling period was about 10% longer at the highest, compared to the lowest, phosphorus regime (Table 3.4). Phosphorus supplied after anthesis reduced the grain filling period in the 1.0 mM P series but extended it in the 0.25 mM P series. While these periods were statistically significantly different the data are for

observations of the glumes and grain, not on the time of cessation of grain dry weight accumulation. The latter, which is examined in Chapter 4, cannot be calculated as accurately as suggested by the data in Table 3.4.

The strongest interactions were seen in the concentrations of phosphorus in vegetative tissues and the concentration of nitrogen in lower leaves. The lower stem was the tissue most sensitive to the phosphorus regime. When the nutrient solution contained 1 mM P stem phosphorus increased from 0.1% P to 0.4% P, as the duration of supply increased (Figure 3.1a), and so had a higher concentration of phosphorus than the inflorescence, but slightly less than in the peduncle. With a 0.25 mM P supply the lower stems did not accumulate significant amounts of phosphorus and the peduncle had a lower concentration than the inflorescence.

The lower leaves contained less phosphorus than the flag leaf in all plants (Figure 3.1b) and those with less than 0.1% phosphorus also had significantly less nitrogen (Figure 3.1c).

The phosphorus and time treatments had independent effects on some plant features (Table 3.4).

The 1 mM phosphorus supply produced larger plants with more tillers per plant. The main culm reached anthesis later and had a slightly longer grain filling period; there were more and heavier grains per ear with a higher concentration of phosphorus (the nitrogen concentrations were similar in grain but higher in vegetative tissues).

Application of phosphorus for a longer time led to more dry matter via continued tiller production, delayed anthesis and extended grain filling, heavier grains, higher yield per ear, and a higher

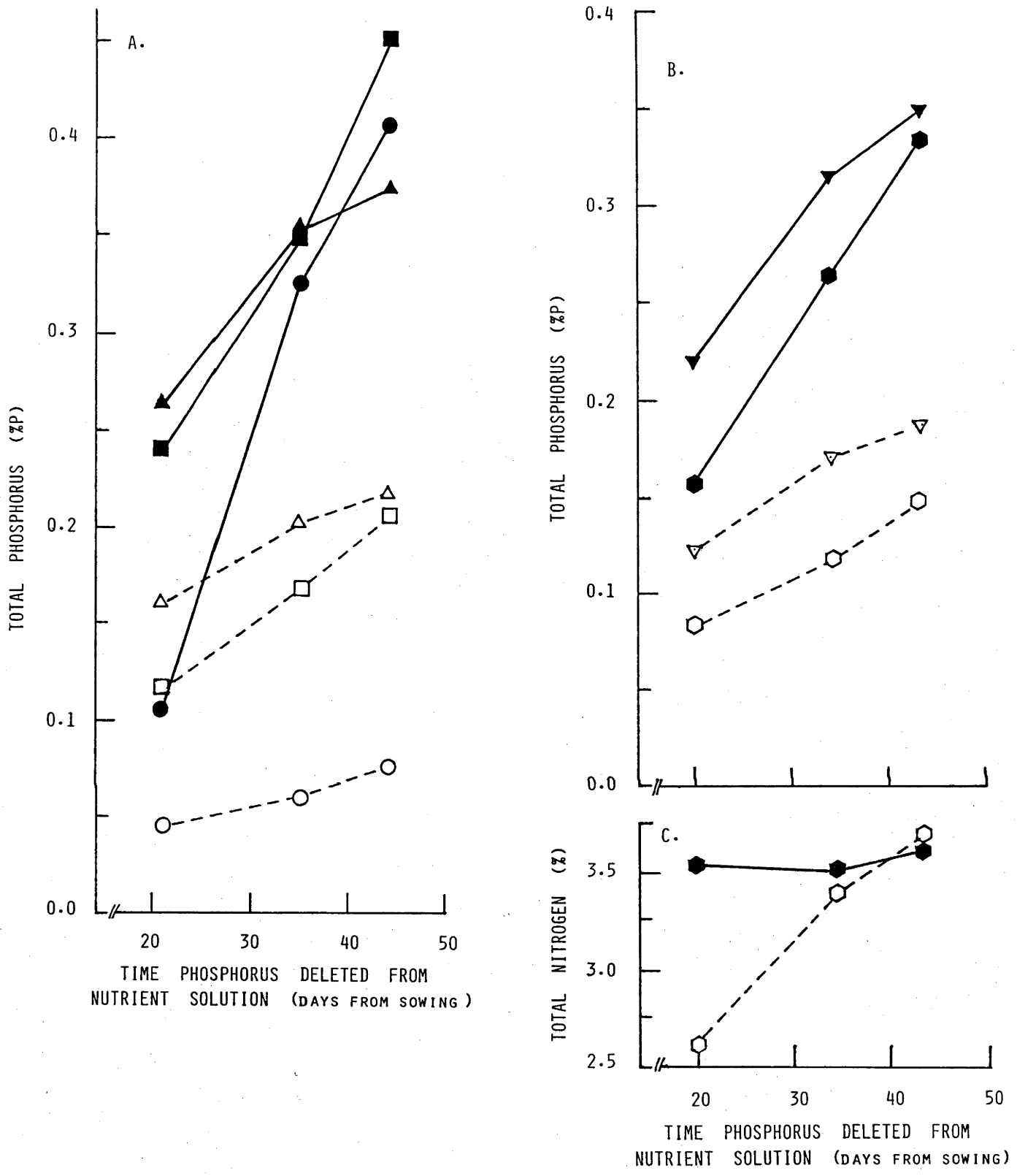


Figure 3.1. Effects of phosphorus regimes on tissue phosphorus (% P) at anthesis, —solid symbols = 1 mM P daily; --- open symbols 0.25 mM P daily when present in nutrient supply.
a) ▲△Inflorescence (*);■□peduncle (**); ● ○ penultimate and lower stem internodes (***).
b) ▼▽flag leaf (n.s.);⬢⬡lower leaves (**); and
c)⬢⬡nitrogen (% N) in lower leaves (**).
n.s., *, **, *** = not a significant interaction and P = .05, .01, .001 respectively.

concentration of phosphorus in all tissues (Table 3.3). Phosphorus applied after anthesis promoted total plant dry matter via new tillers and a slight delay in senescence. The grain yield of the main culm was not increased by continuing the supply of phosphorus to maturity. The number of grains per ear was reduced from 35 to 30 in the 1.0 mM P treatment and this was not offset by the individual grain dry weight. The harvest index values (Table 3.3) indicate that the distribution of dry matter at anthesis was not affected by late applications of phosphorus. So, at either phosphorus level, the early applications of phosphorus were used more efficiently to produce grain than the late applications.

The late applications of phosphorus raised the concentration of phosphorus in the grain from 0.54 to 0.61% and 0.32 to 0.48% for the two levels of application respectively (Table 3.3).

These data suggest that the concentration of phosphorus in the grain was not the factor limiting grain development at either phosphorus supply. It is evidence that the wheat plant can accumulate phosphorus beyond what is required for growth, thus supporting the finding of Piper and de Vries (1964), and the hypothesis that modern wheats can accumulate phosphorus inefficiently. The utilization of late (foliar) applications of phosphorus by plants is further examined in Chapter 6. The accumulation of phosphorus in phytate is examined below.

3.3.2 Experiment 1: Grain phosphorus

The relationship between total and phytate phosphorus in grains is shown in Figure 3.2. The correlation was weak in immature grain but highly linear in mature grain. The weak relationship of the former was not associated with the presence of intermediate esters of

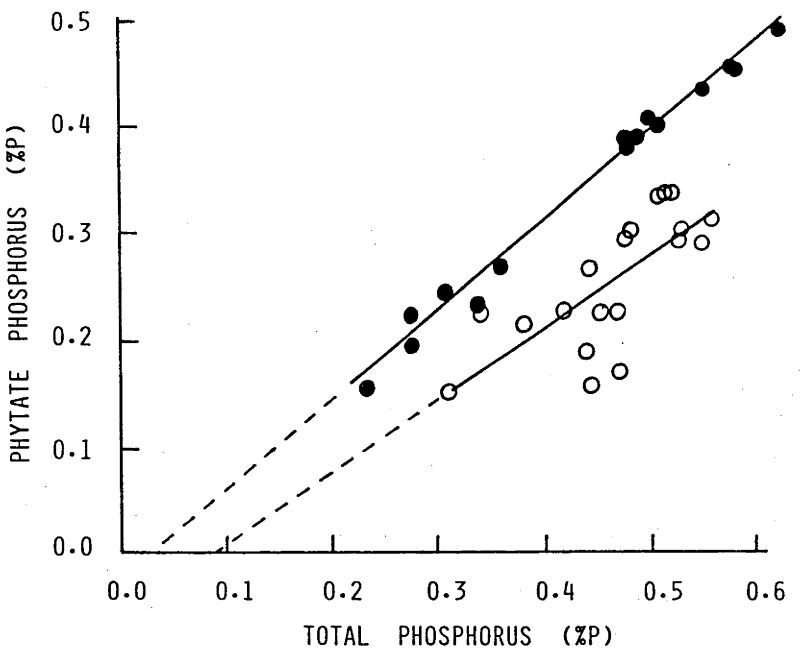


Figure 3.2. Relations between total and phytate bound phosphorus in whole (a) immature (O) and (b) mature (●) wheat grain grown with the phosphorus regimes in experiment 1.
a) Phytate phosphorus (% P) = total phosphorus * 0.672-0.06 (R^2 = 0.522*, n = 19).
b) Phytate phosphorus (% P) = total phosphorus * 0.841-0.025 (R^2 = 0.982***, n = 15).

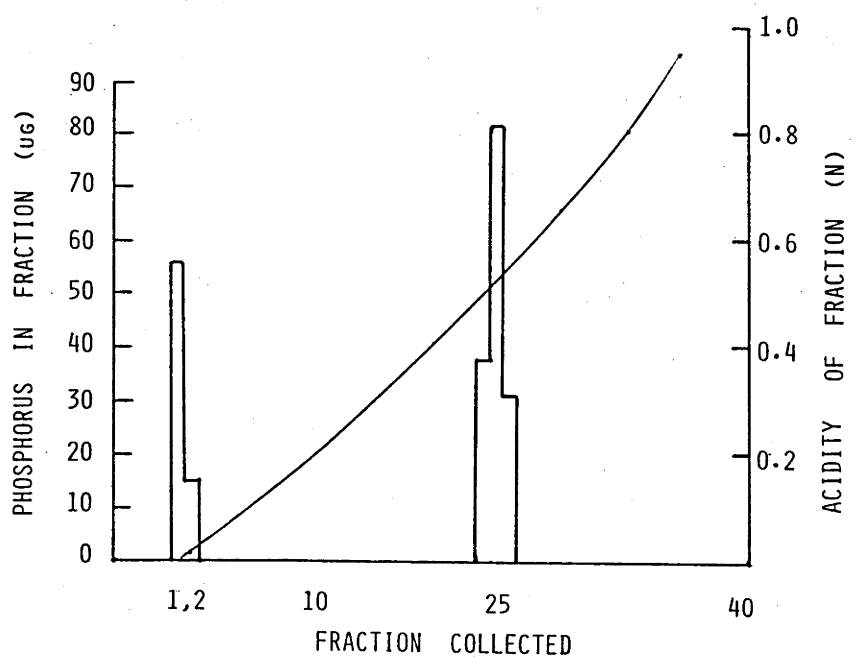


Figure 3.3. Gradient elution of 0.1 M TCA extract of immature wheat grain by increasing the normality of HCl. Bars indicate total phosphorus in each fractions collected. Fractions 1 and 2 contain easily soluble phosphate compounds and fractions 24-26 phosphorus soluble in 0.5N HCl.

myoinositol. The gradient elution used by (Cosgrove, 1980a) showed clearly that only inorganic (plus myoinositol-1-P) and myoinositol-hexakisphosphate were present in the grain (Figure 3.3).

The linearity of the relation for mature grain is in agreement with earlier work (see Chapter 1.2.5). However, the extrapolation of the relationship suggest that when the total mature grain phosphorus level is 0.03% P then no phytate will form. This differs from the values shown in Figure 1.12. Phytate formation is discussed in detail in Chapter 4.

3.3.3 Selection of phosphorus regimes for subsequent studies

Contrasting phosphorus regimes were selected and the distribution of dry matter and phosphorus examined in more detail. The data appear in Appendix 6.

The 1 mM P to maturity regime was chosen as the CONTROL for future studies. This treatment produced the highest yield per plant (although the grain yield potential was probably not achieved as the plants were harvested soon after the main culm reached maturity). It also produced the largest grains, but due to a slight reduction in grains number per ear, the main culm had a lower yield (Table 3.3). This treatment was also a convenient choice because it is the nutrient solution used routinely throughout the Canberra Phytotron. It has been used for many physiological studies there and elsewhere. Both the dry weight and the amount of phosphorus (mg P) in the main culm doubled during grain development (Figure 3.4; Appendix 6.5-6.6).

The regime of 0.25 mM P supplied to the floral initiation stage (20 days) was chosen as the LOW P treatment for most future studies. The plants produced by this treatment are more like plants grown in the field with respect to tiller number per plant, senescence

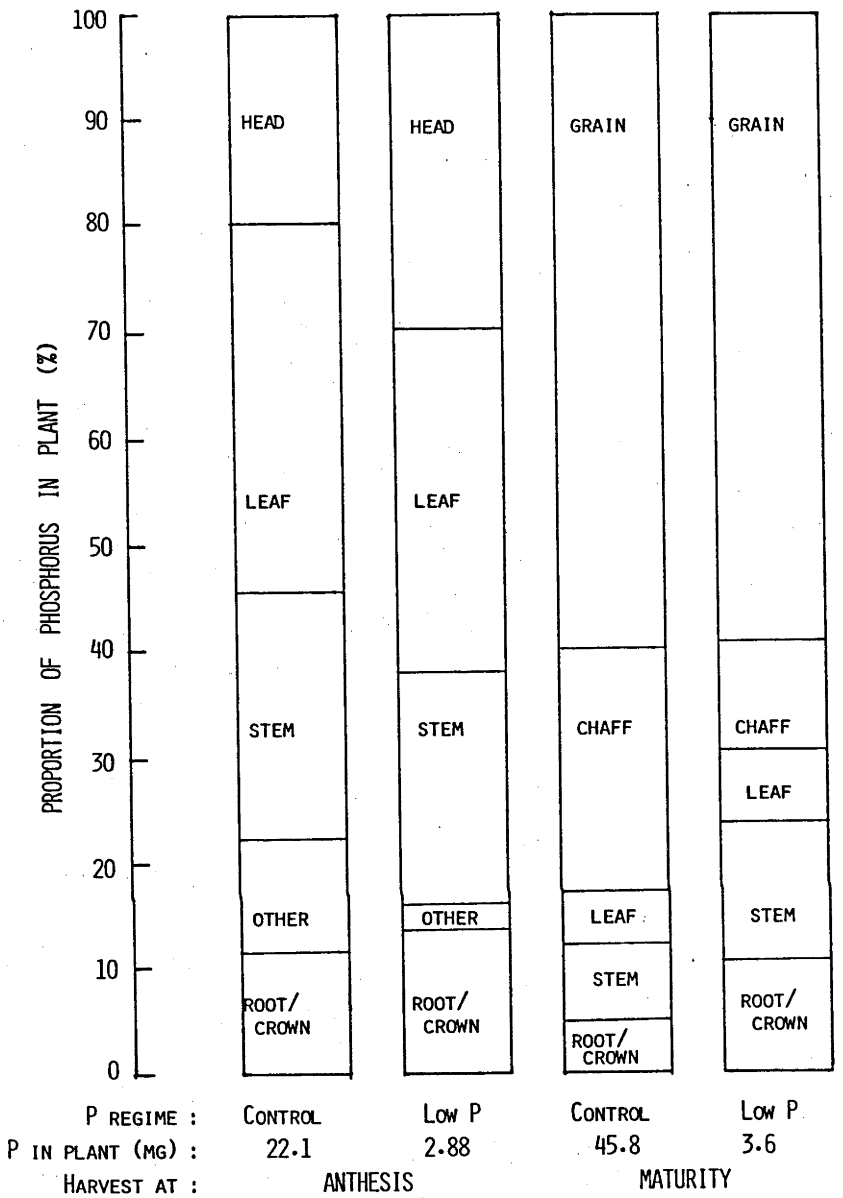


Figure 3.4. Distribution of phosphorus in control and low P plants at anthesis and maturity in plants grown at 24/19°C.

Other = tillers without ears

pattern, grain dry weight and phosphorus uptake. This comparison became more clear when plants were grown at 18/13°C because they took up only 10% of the final phosphorus (mg per plant) after anthesis (Table 3.5) compared to 20.5% when grown here at 24/19°C (Figure 3.4; Appendix 6.5). During grain filling little phosphorus is taken up in the field in Australia (Smith, 1965; Batten and Khan, unpublished). Senescence of tillers and lower leaves was evident near crop maturity (see photos, in Appendix 11). The final grain phosphorus concentration (0.23%, Table 3.4) was similar to levels for modern wheats grown in Australia (Batten et al., 1982).

At maturity the grain from both phosphorus treatments held 62-63% of the phosphorus in the main tiller. In control plants 25% of the phosphorus was in the chaff, 6% in stems and 5% in leaves, while low P plants had only 11% in chaff, but 18% in stems and 9% in leaves (Appendix 6.6). Figure 3.4 presents the distribution of phosphorus in whole plants.

3.3.4 Experiment 2: Effects of temperature regimes

Plants grown at lower pre-anthesis temperatures took longer to reach anthesis were taller, heavier, had larger flag leaves, and significant for this study, lower concentrations of phosphorus (Table 3.5a). Plants grown at 24°/19°C took longer to reach anthesis and produced more dry weight by anthesis than would have been predicted from the trends across the other temperatures. This suggests that the plants grown in the 24/19° glasshouse were in a 'cool' spot within that glasshouse. In the first seven days after anthesis, i.e. at 18°/13°C, all plants grew by 5-8 cm, then ceased elongating.

Plants grown at low pre-anthesis temperatures began to senesce before anthesis while those grown at higher temperatures remained completely green for two to three weeks after anthesis (see photographs in Appendix 11).

The net carbon exchange rate by the flag leaf was significantly lower at anthesis and declined more rapidly thereafter, than did the rate for plants from warmer treatments (Figure 3.5). The plants grown at 30°/25°C prior to anthesis had a net carbon exchange rate which for the first 25 days of grain filling approached that of Control plants (1 mM P to maturity) (cf.Chapter 4, Figure 4.5).

Figure 3.6a shows the relationship between photosynthesis and leaf phosphorus. As the leaf total phosphorus (%) fell below about 0.15% the N.C.E. rate declined sharply. In this experiment the relationship was not improved by replacing total phosphorus by soluble phosphorus. At anthesis 25% of the phosphorus in the flag leaf after each treatment (Table 3.5a) was extracted by the sulphuric acid method of Bouma and Dowling (1982). This technique measures inorganic phosphorus almost exclusively (Irving and Bouma, 1984). Similar phosphorus levels (data not presented here) were obtained by extracting with 2% acetic acid (Johnson and Ulrich, 1959).

Figure 3.6b and c show that leaf chlorophyll and leaf total nitrogen also declined rapidly as the leaf phosphorus fell below 0.15% P. Therefore leaf nitrogen was linearly related to photosynthesis (Figure 3.6d).

The relationship between leaf nitrogen and phosphorus for these low P plants is similar to that for nitrogen deficient plants reported by Evans (1983) who found that the leaf nitrogen content was approximately proportionally related to chlorophyll content and rubisco

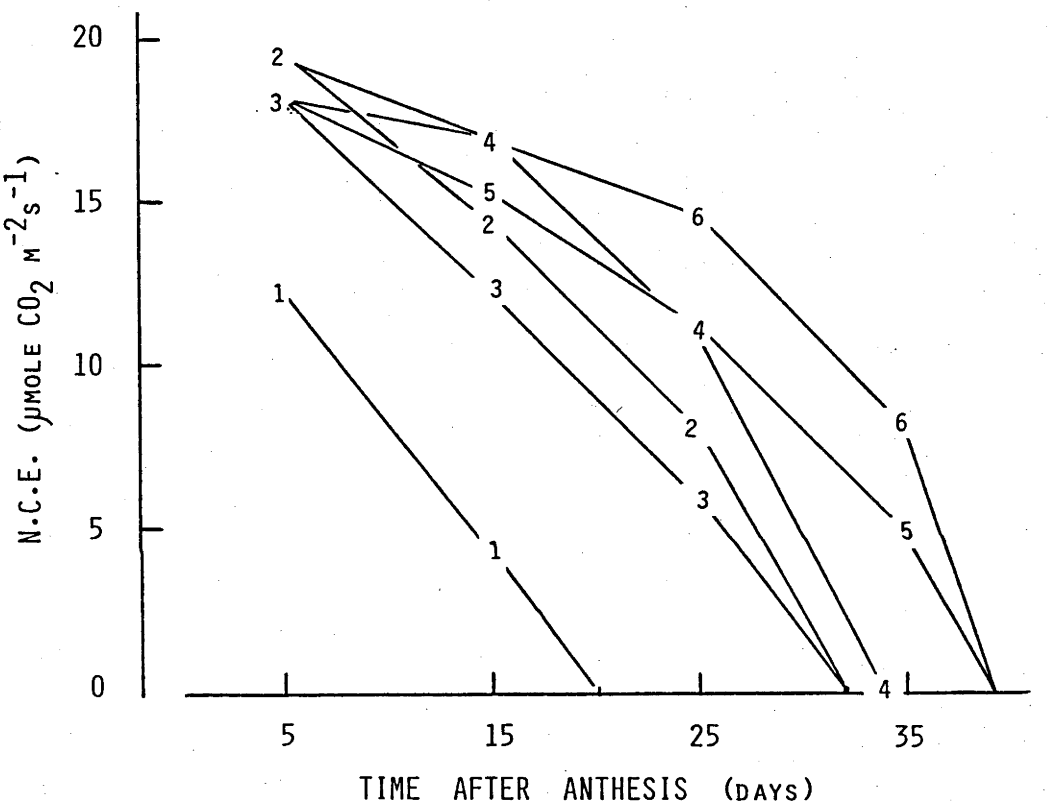


Figure 3.5. Photosynthesis (N.C.E.) of the flag leaf during grain development in plants grown at six preanthesis temperatures, vis. 1 15/10°, 2 18/13°, 3 21/10°, 4 24/19°, 5 27/22° and 6 30/25°C.

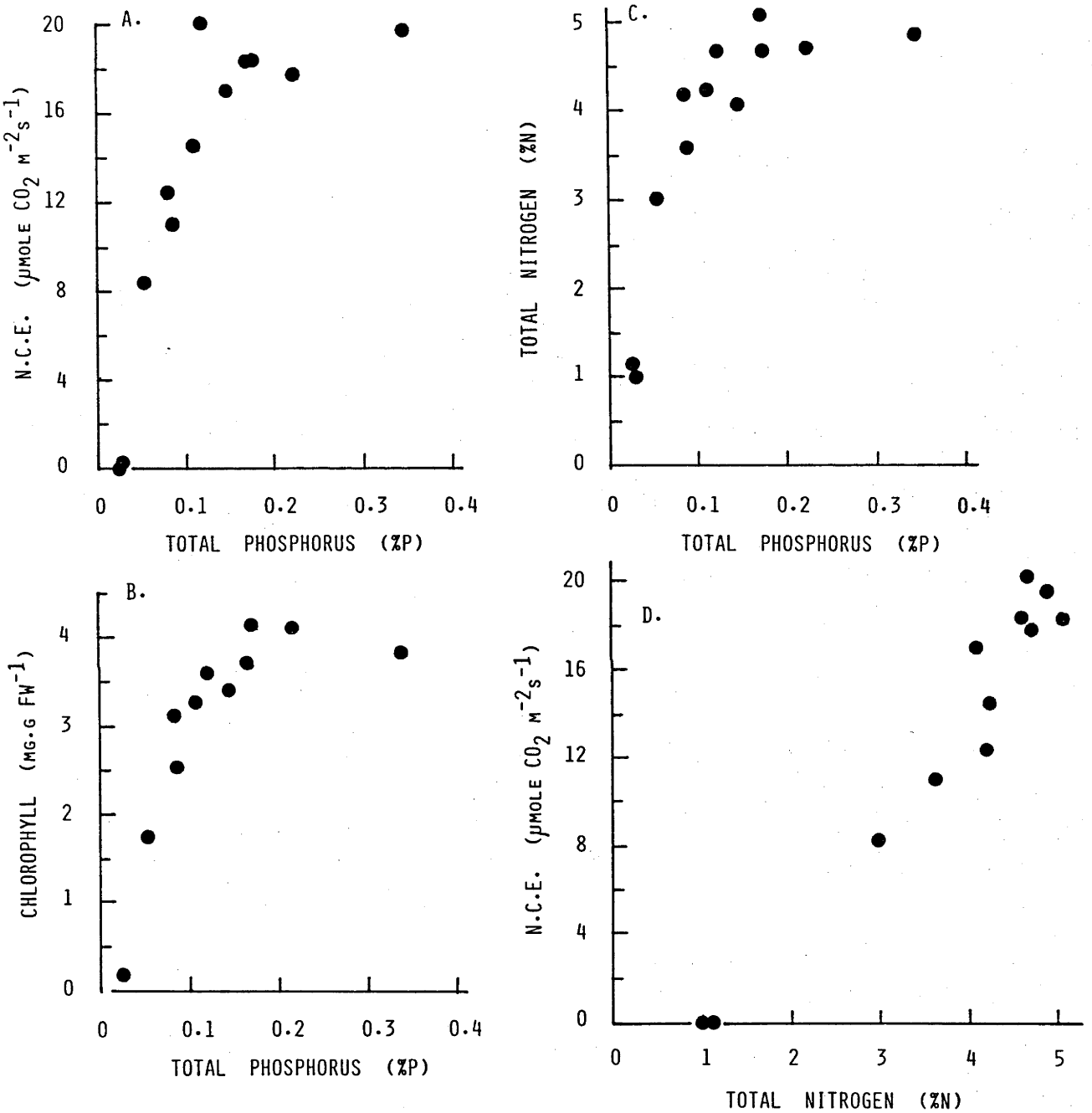


Figure 3.6. Relations between photosynthesis (N.C.E.) phosphorus, nitrogen and chlorophyll of the flag leaf during grain development of low P plants grown at six preanthesis temperatures.

activity. The leaf nitrogen levels, which were induced by the export of phosphorus, are therefore the dominant factor causing leaf death . This conclusion is supported by data from another series of senescing leaves (see Appendix 7) which showed that the nitrogen concentration declined faster than the phosphorus concentration as the leaf lost chlorophyll.

During the grain development period (45-54 days) there were larger dry matter increases by plants grown at higher pre-anthesis temperatures. This was due to continued production of tillers in the plants which had higher tissue phosphorus levels and were not dominated by senescence. The dry weight of main culms increased by 42 and 90% whereas the total plant weight increased by 31% and 34% over anthesis dry weights for plants from 15/10 and 30/25° pre-anthesis temperatures respectively. The additional growth of the latter was associated with the uptake of more phosphorus both before and after anthesis (Table 3.5b). Because the grain yield of the main culm ear was less affected by the pre-anthesis treatments the concentration of phosphorus varied from 0.16 to 0.31%.

Grains in upper spikelets in the ear, and in the outer florets of central spikelets, were consistently lighter with lower concentrations of phosphorus and nitrogen (Table 3.6). This indicates that unloading of assimilate and nutrients from the phloem is biased in favour of the grains closest to the rhachis, and the peduncle. The variations in grain % N for low P plants are similar in magnitude to those for control plants grown by Bremner (1972). This suggests that growth and nutrient content of the grains is affected by the sink (the vascular connections in the ear) more than by the supply of nutrients from the sink.

The distribution of phosphorus in the plants at maturity is shown in Figure 3.7. Plants from the lower pre-anthesis treatments has less phosphorus in the grain (i.e. a lower phosphorus harvest index) and a significant proportion in leaves and other (non ear bearing and senescing tillers). Although the phosphorus harvest index rose with increasing pre-anthesis temperature treatments, the proportion in roots remained at about 10%.

Phosphorus and nitrogen were both exported from the flag leaf during anthesis as photosynthesis declined (Figures 3.3 and 3.8). The export of nitrogen was more rapid than that of phosphorus during the chlorophyll destruction phase (i.e. 15 to 27 days after anthesis for treatment 2; or 25 to 35 days after anthesis for treatment 4 in Figure 3.8).

The suggestion by Williams (1948), that senescence may be due to export of nitrogen rather than phosphorus is similar but not directly comparable. In that study the highest level of phosphorus applied (P3) was 120 mg P plant^{-1}. This produced plants with 1.87 and 2.27% P in leaf dry matter in the first and second mature leaves respectively. These concentrations in wheat could produce toxic symptoms (Appendix 5) and depress photosynthesis (Cartwright et al., 1974). Williams (1948) noted a depression in dry matter gain in the P3 plants which were also nitrogen deficient, senesced rapidly after anthesis and took up no phosphorus after anthesis.

From these plants Williams (1948) calculated that ".... phosphorus deficient plants derived only 30 per cent of their inflorescence phosphorus from other plant parts, whereas those plants which had an excessive supply derived no less than 93 per cent of their inflorescence phosphorus from these sources." These figures

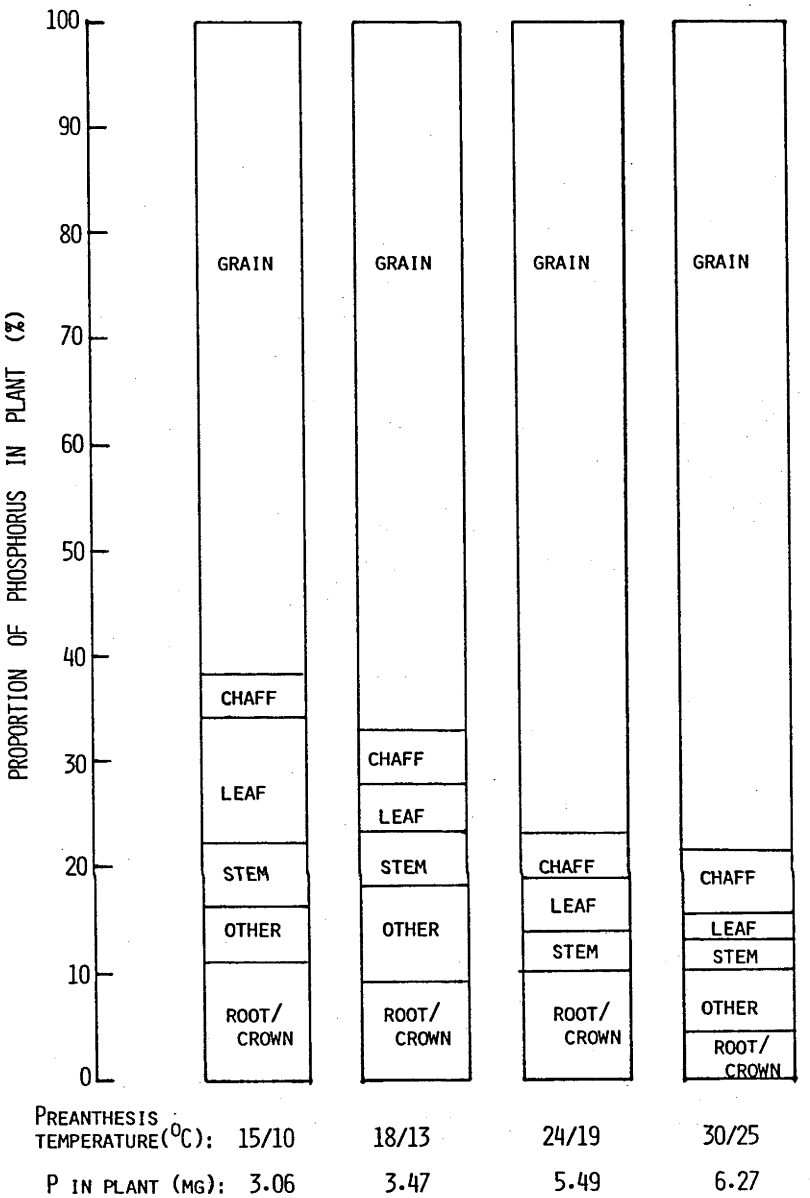


Figure. 3.7. Distribution of phosphorus in low P plants grown at four preanthesis temperatures then at 18/13°C during grain development. Other = tillers without ears

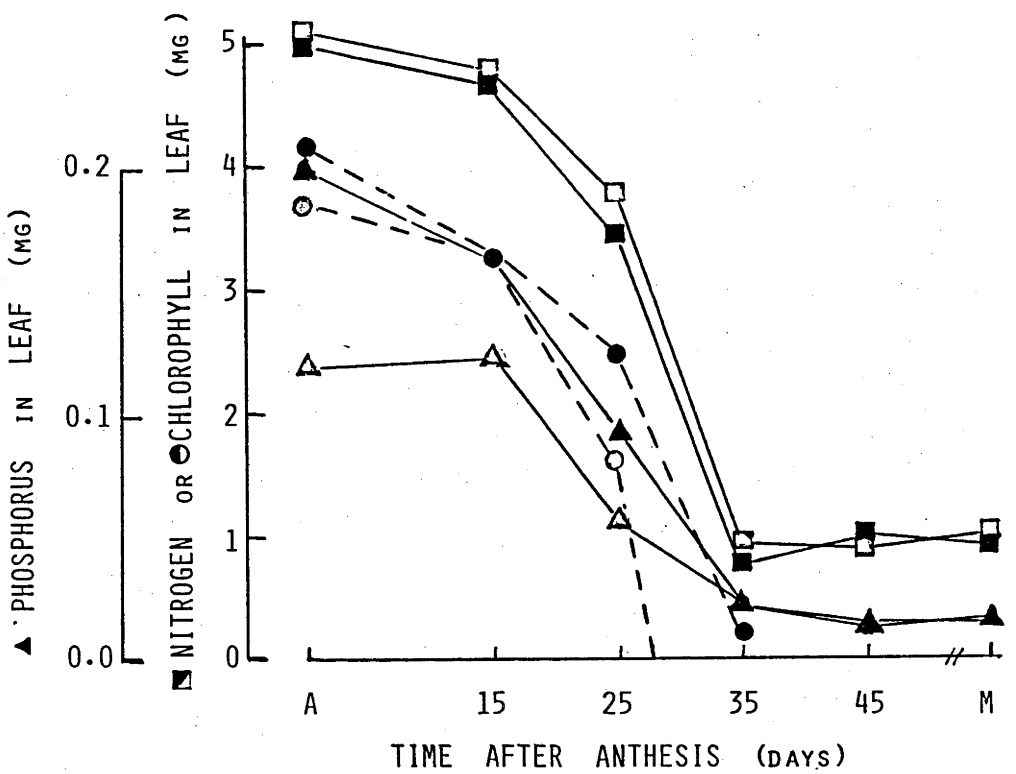


Figure 3.8. Phosphorus, nitrogen and chlorophyll (mg) in the flag leaf blade after anthesis in plants grown two preanthesis temperatures - 18/13° (□) and 24/19° (■).

○ ●

△ ▲

have been cited regularly as evidence of the degree of mobility of phosphorus in cereals.

Data from experiment 1 (Appendix 6.5-6.6) and experiment 2 (Appendix 6.9-6.10) were used to calculate the proportion of phosphorus in the grain which was derived from other plant tissues. Control plants grown at 24/19°C derived 21% of their grain phosphorus from shoot tissue (other than the chaff), while in low P plants the amount derived from other tissues ranged from 51 to 89% with 6 to 12% coming from the flag leaf blade (Table 3.7). Therefore, given a non-limiting supply of nitrogen a plant with adequate phosphorus can produce grain without a large net transfer of phosphorus from leaves and stems. On the other hand, given a low phosphorus supply after anthesis, a wheat plant will derive the majority of its grain phosphorus from other shoot tissues.

3.4 CONCLUSIONS

The various phosphorus levels, times of supply of phosphorus, and pre-anthesis temperature treatments produced a wide range in plant dry matter and phosphorus levels and distributions.

Control and Low P regimes have been selected for use in subsequent studies. Although some phosphorus was taken up during grain development by plants on a low P regime, the phosphorus concentration of grain can be lower than expected for field grown wheat. By contrast, grains of control plants have higher concentrations of phosphorus than usually found in the field in Australia (Williams and Colwell, 1977).

It must be stressed that the plants produced by control and low P regimes differ significantly in ontogeny. Control plants, and low P

plants grown at high pre-anthesis temperatures, maintained leaves in a green state until after the heads had senesced (a situation which is not normally seen in the field). Low P plants grown at lower pre-anthesis temperatures progressively senesced from the older leaves up to the ear (as shown in photos in Appendix 11).

Table 3.1 The phosphorus regimes used in experiment 1

PHOSPHORUS (Concentration when present in nutrient solution (mM P)	TIME Plant growth stages when phosphorus deleted from the nutrient solution (days after sowing)*			
	Floral initiation	Flaf leaf emergence	Anthesis	Maturity
1.0	21	35	45	82 (CONTROL)**
0.25	21 (LOW P)**	35	43	77

* Growing at 24°C day/10° night.

**Phosphorus x Time combinations used in most subsequent studies in this thesis.

Table 3.2 The relations between phosphorus regimes and total plant dry matter at anthesis (a) and maturity (b) (g)

	Plant growth stage when phosphorus was deleted from the nutrient solution			
a) Anthesis				
Time	Floral initiation	Flag leaf emergence	Anthesis	Maturity
Phosphorus (mM P)				
1.0	4.00	4.61	4.47	
0.25	2.11	2.67	2.73	
s.e.		not significant		
b) Maturity				
1.0	8.80	12.36	14.32	17.62
0.25	4.06	5.94	6.49	9.50
s.e.		0.76*		

Table 3.3 Data to show the effects of the phosphorus regimes on plant features at maturity

Time	Floral initiation	Flag leaf emergence	Anthesis	Maturity
Phosphorus (mM P)				
Whole plant				
Grain yield (g plant^{-1})				
1.0	2.7	4.2	4.9	4.7
0.25	1.0	1.6	2.2	2.2
s.e.: phosphorus 0.2***; time 0.3***				
Shoot:root ratio				
1.0	9.5	9.5	9.9	8.4
0.25	4.9	7.2	8.2	6.3
s.e.: phosphorus 0.3***; time 4.7**; phosphorus x time 0.7*				
Non ear bearing tillers at maturity				
1 mM	1	2	3	5
0.25 mM	-1^{+}	0	0	4
Main culm only				
head dry weight (g)				
1.0	1.1	1.4	1.6	1.5
0.25	0.9	1.0	1.1	1.2
s.e.: phosphorus 0.06***; time 0.08**				
grain yield (g ear^{-1})				
1.0	0.76	0.94	1.17	1.07
0.25	0.58	0.64	0.72	0.80
s.e.: phosphorus 0.05***; time 0.08**				
grain weight (mg grain^{-1})				
1.0	28	33	34	35
0.25	27	28	28	32
s.e.: phosphorus 0.7***; time 0.9***				
Grains ear^{-1}				
1.0	27	28	35	30
0.25	22	23	26	25
s.e.: phosphorus 1.7**				

Table 3.3 contd.

Grain phosphorus (% DM)				
1.0	.37	.49	.54	.61
0.25	.23	.28	.32	.48
s.e.: phosphorus 0.01***; time 0.02***				
Main culm as a proportion of total, shoot dry weight (%)				
1.0	28	24	25	24
0.25	60	42	35	36
s.e.: phosphorus 2.0***; time 2.4***; phosphorus x time 3.5***				
Main culm grain yield as a proportion of total plant grain yield (%)				
1.0	28	23	24	23
0.25	59	42	33	39
s.e.: phosphorus 2.4***; time 3.5**; phosphorus x time 4.9*				
Main culm harvest index (grain x 100/shoot)				
1.0	36	38	43	41
0.25	30	31	36	37
s.e.: phosphorus 1.5***; time 2.1*				

[+]Indicates a senescing tiller

Table 3.4 Effects of phosphorus regimes on the number of days to anthesis and on the grain filling period

(a) Days to anthesis	Plant growth stage when phosphorus was deleted from the nutrient solution			
Phosphorus (mM P)	Floral initiation	Flag leaf emergence	Anthesis	Maturity
1.0	42	43	44	46
0.25	41	42	42	43
s.e.	phosphorus 0.7*; time 1			
(b) Days of grain filling				
1.0	32	34	37	36
0.25	32	33	33	34

s.e.: phosphorus 0.3***; time 0.4***; phosphorus x time 0.6***

Table 3.5 Effects of pre-anthesis temperature treatments on plant growth and phosphorus (Experiment 2)

a. AT ANTHESIS								
Pre-anthesis temperature (°C, day/night)		15/10	18/13	21/16	24/19	27/22	30/25	s.e.
WHOLE PLANT								
dry matter	(g)	3.72	1.88	1.90	2.80	2.03	1.70	0.24
tillers		2.7	2.1	2.7	3.0	3.7	2.9	0.4
ears		1.2	1.1	1.0	1.4	1.2	1.0	0.2
shoot:root ratio		3.1	3.1	3.2	3.9	3.9	4.3	0.2
total phosphorus	(mg)	2.73	1.58	n.a.	3.3	n.a.	4.02	
MAIN CULM								
height	(cm)	58	50	49	46	41	37	2
anthesis (days after sowing		68	53	46	50	43	42	1.5
culm dry weight	(g)	1.9	1.2	1.2	1.2	0.9	1.0	0.1
head dry weight	(g)	0.36	0.28	0.30	0.30	0.24	0.27	0.02
FLAG LEAF BLADE								
area	(cm^2)	28	22	21	21	18	13	2
dry weight	(g)	0.19	0.11	0.10	0.11	0.11	0.08	0.01
chlorophyll	(mg.g FW^{-1})	3.1	3.6	3.7	4.2	4.1	3.8	0.1
phosphorus	(%DM)							
total		0.09	0.12	0.17	0.17	0.22	0.34	0.02
soluble		0.02	0.03	0.04	0.04	0.06	0.12	0.002

na = not analysed

Table 3.5 contd.

b. AT MATURITY

		15/10	18/13	21/16	24/19	27/22	30/25	s.e.
WHOLE PLANT								
shoot dry matter	(g)							
total shoot		4.1	3.0	2.8	5.3	5.7	6.6	0.8
mature shoot[2]		3.7	2.8	2.6	5.0	4.5	4.0	0.6
grain	(g)	1.4	1.1	1.0	1.8	1.7	1.8	0.2
tillers		2.0	2.0	1.4	2.8	3.9	4.8	0.6
root dry matter	(g)	0.78	0.60	0.54	0.85	0.82	0.89	0.11
phosphorus	(mg)	3.06	3.25	na	5.19	na	6.90	na
MAIN CULM								
height	(cm)	65	56	57	56	47	43	2
anthesis (days after sowing)		67	52	48	48	47	42	1.4
dry weight	(g)	2.7	2.4	2.3	2.2	1.8	1.9	0.1
fertile spikelets		14	13	13	14	12	14	1
grain yield	(g)	1.0	0.95	0.92	0.87	0.73	0.95	0.07
grain number		37	33	31	29	24	32	2
phosphorus	(mg)	2.09	2.34	na	2.55	na	3.27	na
CHANGES DURING GRAIN FILLING								
dry weight gains (g. plant^{-1})		1.18	2.42	1.40	3.30	4.47	5.20	na
phosphorus gains (mg. plant^{-1})		0.33	1.66	na	1.86	na	2.88	na

na = not assessed.

(1) Standard errors based on analysis of data which assumed that temperature effects are not confounded by glasshouse effects.

(2) Shoot excluding tillers which formed after anthesis of the main culm.

TABLE 3.6 Dry weight and concentration of phosphorus and nitrogen in grain at different positions of the spike and spikelet

(Experiment 2)

Preanthesis temperature (°C, day/night)	15/10	18/13	21/16	24/19	27/22	30/25	s.e.
DRY WEIGHT (mg.grain^{-1})							
Upper spikelets	22.7	23.7	25.1	24.7	26.9	27.3	1.2
4 central spikelets							
A and B florets	32.5	33.3	33.5	33.7	32.8	33.6	1.1
C and D florets[1]	26.2	26.2	26.3	29.9	25.6	24.1	1.2
Lower spikelets	29.7	31.1	32.1	32.6	30.5	30.8	1.2
PHOSPHORUS (% DM)							
upper spikelets	0.13	0.15	na	0.19	na	0.27	na
4 central spikelets							
A and B florets	0.15	0.18	0.19	0.22	0.25	0.30	0.01
C and D florets	0.13	0.17	na	0.20	na	0.28	na
lower spikelets	0.15	0.19	na	0.24	na	0.30	na
NITROGEN (% DM)							
upper spikelets	2.98	2.98	na	3.11	na	2.97	na
4 central spikelets							
A and B florets	3.40	3.35	na	3.50	na	3.36	0.11
C and D florets	3.05	3.08	na	3.29	na	2.84	na
lower spikelets	3.29	3.00	na	3.58	na	3.25	na

1. only 0.6 to 1.3 grains per 2 (C+D) florets

na = not analysed

TABLE 3.7 Summary of translocation of phosphorus from other tissues to the grain under different treatments

Phosphorus regime	Temperatures (°C)	Proportion (%) phosphorus in grain derived from		
		Other shoot tissue(1)	Flag leaf	
			blade	sheath
Control	24/19 throughout	21	9	
Low P	24/19 throughout	58	15	
Low P	15/10 preanthesis 18/13 post anthesis	77	10	9
Low P	18/13 throughout	51	6	8
Low P	24/19 preanthesis 18/13 post anthesis	71	11	9
Low P	30/25 preanthesis 18/13 post anthesis	89	12	11

(1) Root/crown phosphorus content did not change appreciably and was not included in the calculations. At low temperatures where tillers senesced after anthesis the proportion derived from other tissues is possibly slightly underestimated.

CHAPTER 4

GRAIN DEVELOPMENT AND SENESCENCE IN RELATION TO CHANGES IN PHOSPHORUS

4.1 INTRODUCTION

4.2 MATERIALS AND METHODS

4.2.1 Phosphorus regimes

4.2.2 Sampling and measurements

4.2.2.1 Senescence

4.2.2.2 Grain growth

4.2.2.3 Endosperm cell number

4.2.2.4 Phosphorus determinations

4.2.2.5 Other

4.3 RESULTS

4.3.1 General plant growth and senescence

4.3.2 Grain growth

4.3.3 Plant phosphorus

4.4 DISCUSSION

4.4.1 Grain development in relation to senescence

4.4.2 Senescence in relation to phosphorus

4.4.3 Grain development in relation to phosphorus

4.1 INTRODUCTION

The results presented in Chapter 3 showed that wheat plants grown with a limited supply of phosphorus exhibit early senescence of the vegetative tissues, have a shorter grain filling period and produce fewer and lighter grains than plants grown with adequate phosphorus.

These differences prompt the questions:

1. Is leaf senescence related to exhaustion of a particular form of phosphorus (e.g. the level of inorganic phosphorus)?
2. Do low P plants produce lighter grains because the rate of grain filling, the duration of grain filling, or both these factors, is reduced under phosphorus stress?
3. What effect does early senescence of the photosynthetic system have on grain filling?, and
4. Does the supply of phosphorus to the grain influence grain development directly?

The aim of the experiment reported in this Chapter was to quantify and integrate the patterns of senescence and grain filling in plants under contrasting phosphorus regimes. Attention was concentrated on two aspects of plant phosphorus. Firstly, the levels of ester, inorganic, lipid and residue phosphorus in leaf tissue were measured during grain development and secondly, the accumulation of different forms of phosphorus in the grain.

There are numerous reports of different levels of phytate in mature grain when plants are grown with different phosphate treatments (Chapter 1.). Jennings and Morton (1963) dissected wheat grains grown in the field and found that rapid synthesis of phytate coincided with a decline in both inorganic phosphorus and the loss of

water from the endosperm and the testa-pericarp. However both Rijven (1964) and Williams (1970) concluded that the endosperm (if carefully dissected) contains no phytic acid. This has been confirmed by X-ray microprobe analysis (Tanaka et al., 1974a,b).

In all of the above studies plants were grown at one level of phosphorus but Asada and Kasai (1959) cultured rice with 0.32 or 0.16 mM P. Plants grown with the higher supply of phosphorus accumulated more phosphorus as phytate while inorganic, acid insoluble and ester phosphate were not appreciably affected.

If phytate only forms in grain when the level of inorganic phosphorus is higher than required for metabolism (Chapter 1.3.6), and if translocation of phosphorus into the grain is a limiting factor, then no phytate should be formed in the grain of a phosphorus deficient plant. During the period of rapid water loss prior to maturity (Sofield et al., 1977b) the concentration of inorganic phosphorus may increase without the formation of phytate if the grain is metabolically inactive.

4.2 MATERIALS AND METHODS

4.2.1 Phosphorus regimes

Wheat (cv. Kite) plants were grown as described in Chapter 2 in a glasshouse controlled to 18°C during the day and 13°C at night. Two phosphorus regimes were used. The control plants received nutrient solution which contained 1 mM P through to maturity. Low P plants were produced by applying nutrient solution which contained 0.25 mM P for 18 days and no phosphorus thereafter. The plants of these two treatments were maintained on adjoining trolleys in the glasshouse.

4.2.2 Sampling and Measurements

4.2.2.1 Senescence

Three measurements of senescence were taken on the flag leaf. At each sampling the proportion of the flag and lower leaf area still photosynthetic (green) was estimated by eye. A disc (10 mm) was taken from the leaf (Figure 4.1) weighed immediately then stored at -25°C in the dark until analysed for chlorophyll, phosphorus and nitrogen contents (Chapter 2).

The net carbon exchange of the flag leaf (Chapter 2.5.5) was measured at weekly intervals on the same set of plants. The repeated handling of the leaves resulted in only negligible damage. The response of the leaves to light was recorded at several plant ages.

4.2.2.2 Grain growth

At each sampling time 4 grains were taken from the A or B florets of central spikelets of three plants in each treatment. These were weighed immediately then frozen in liquid nitrogen and dried to constant weight under vacuum.

4.2.2.3 Endosperm cell number

Grains were taken from the basal floret of a central spikelet of 12 control and 14 low P plants 22 days after anthesis (i.e. when maximum cell number was expected; Gleadow et al., 1982; Wardlaw, personal communication). The pericarp was removed and the starchy endosperm fixed in FAA (300 ml 95% ethanol:120 ml water:25 ml acetic acid:25 ml formalin) and stored in 70% ethanol. After transfer to water the cell nuclei were stained using Feulgen reagent then counted following the procedure of Rijven and Wardlaw (1966).

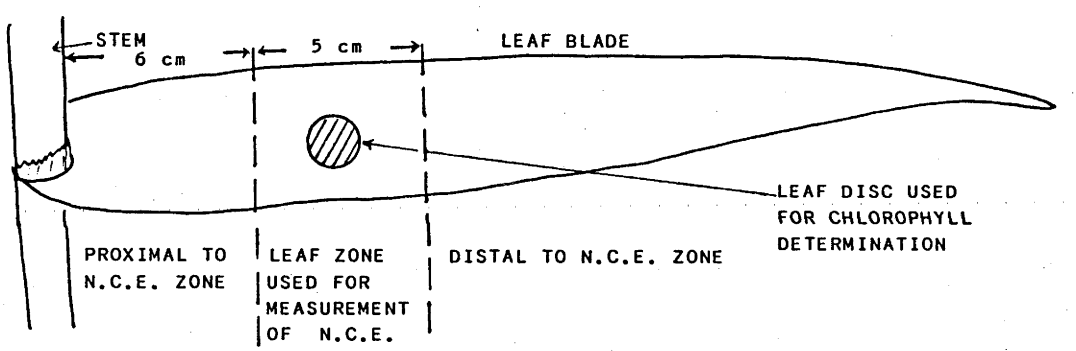


Figure 4.1 Diagram showing the technique used for sampling the flag leaf.

4.2.2.4 Phosphorus determinations

The portion of the leaf within the leaf chamber when measuring net carbon exchange (Figure 4.1) was weighed fresh, frozen in liquid nitrogen, dried under vacuum and later analysed for inorganic, ester, lipid and residue phosphorus fractions as described in Chapter 2.7.2.

The grain samples were analysed for inorganic + ester, phytate and residue phosphorus as described in Chapter 2.7.3.

4.2.2.5 Other

The remainder of each plant was also freeze-dried and retained for stem internode dry weight.

4.3 RESULTS

4.3.1 General plant growth and senescence.

The low P plants produced few tillers, were shorter, reached anthesis three days earlier, had fewer spikelets with some infertile spikelets and also smaller flag leaves than the control plants (Table 4.1).

Stem dry weights, which were strongly influenced by plant height, increased during the early grain filling period. The weight of the peduncle increased rapidly in the control plants and by seven days after anthesis was heavier than the penultimate stem internode. In low P plants the peduncle remained lighter than the penultimate stem internode (Figure 4.2).

Visual assessments

The leaves and peduncle of control plants remained green until the final harvest 80 days after anthesis. On low P plants the flag leaf and the leaf below it (F-1) senesced rapidly after anthesis (Figure 4.3). Low P plants lost chlorophyll from glumes and grain

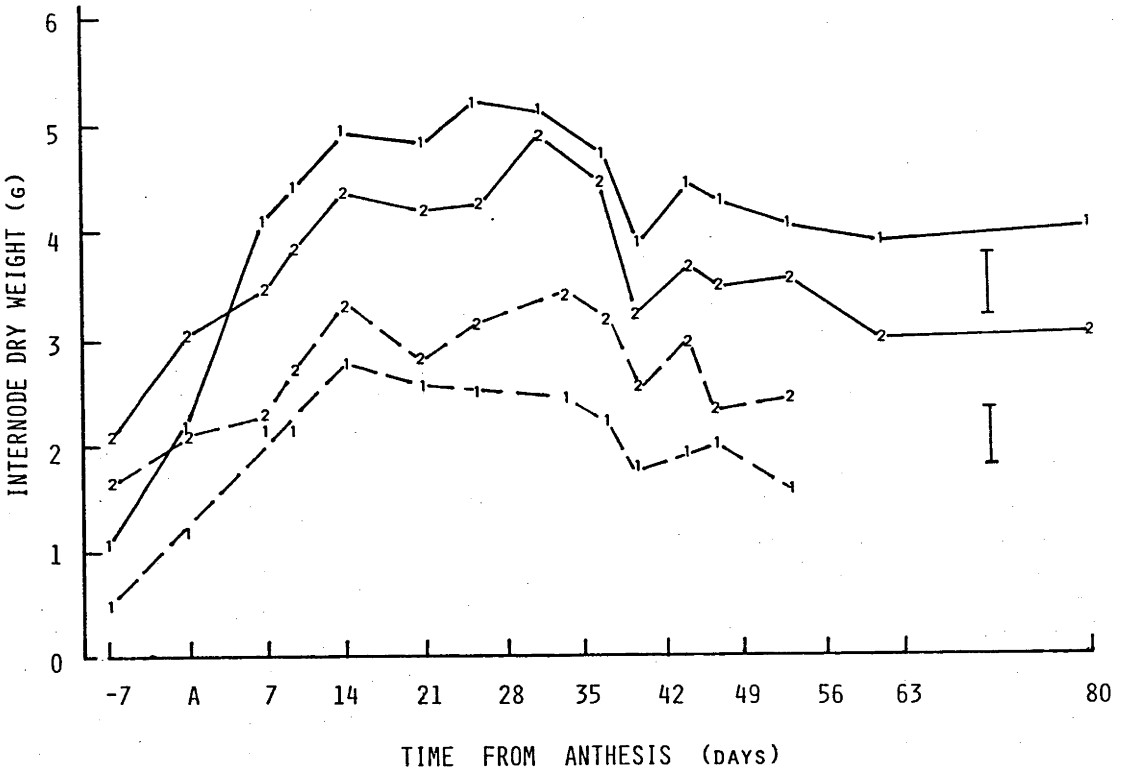


Figure 4.2 Dry weight of peduncle (1) and penultimate internode (2) during the grain development stage in control (——) and low P plants (---).
Each data point is the mean of three replicates.
Vertical bars indicate the 2x the average standard error of the means.

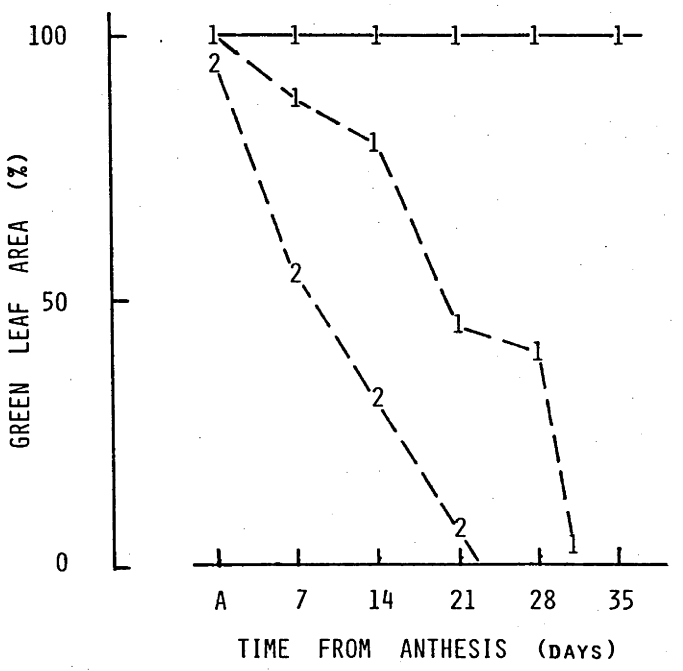


Figure 4.3 Visual assessment of leaf senescence during the grain development stage; 1——1, control flag leaf, 1--1, low P flag leaf, 2--2 low P penultimate leaf.

and the grains became harder 6-10 days earlier than in control plants (Table 4.2).

Leaf chlorophyll

The flag leaf of low P plants had less chlorophyll per unit leaf area and per unit fresh weight and the levels declined rapidly 14 days after anthesis as the "senescence front" moved through the central zone of the leaf (Figure 4.1). There was a decline in chlorophyll in the flag leaf of control plants although there was no visible change in greenness (Figure 4.3 and 4.4). The ratio of chlorophyll a/b remained constant at 2.30±0.08 to 61 days after anthesis. In low P flag leaves the ratio was the same as the control for 14 days after anthesis and then declined to 1.6±0.6 at 26 days after anthesis (Figure 4.4c).

Net carbon exchange (N.C.E.)

The photosynthetic rate of the control flag leaf declined from 24 μmole m^{-2} s^{-1} at anthesis to 17 μmole m^{-2} s^{-1} 49 days after anthesis and to 8 μmole m^{-2} s^{-1} at the final harvest. The low P plants had a significantly lower N.C.E. at anthesis and this declined to a net loss of CO_2 25 days after anthesis (Figure 4.5). Note: this reading is for the centre of the leaf (Figure 4.1).

The N.C.E. rates were measured at different photon flux densities four (control) or three (low P) times during grain development as shown in (Figure 4.6). During the early grain filling period control plants responded to the highest photon flux density available (1100 μmole m^{-2} s^{-1}) but 83 days after anthesis when the plants had mature heads, the flag leaf did not show a significant increase in N.C.E. when the photon flux density exceeded 750 μmoles m^{-2} s^{-1}.

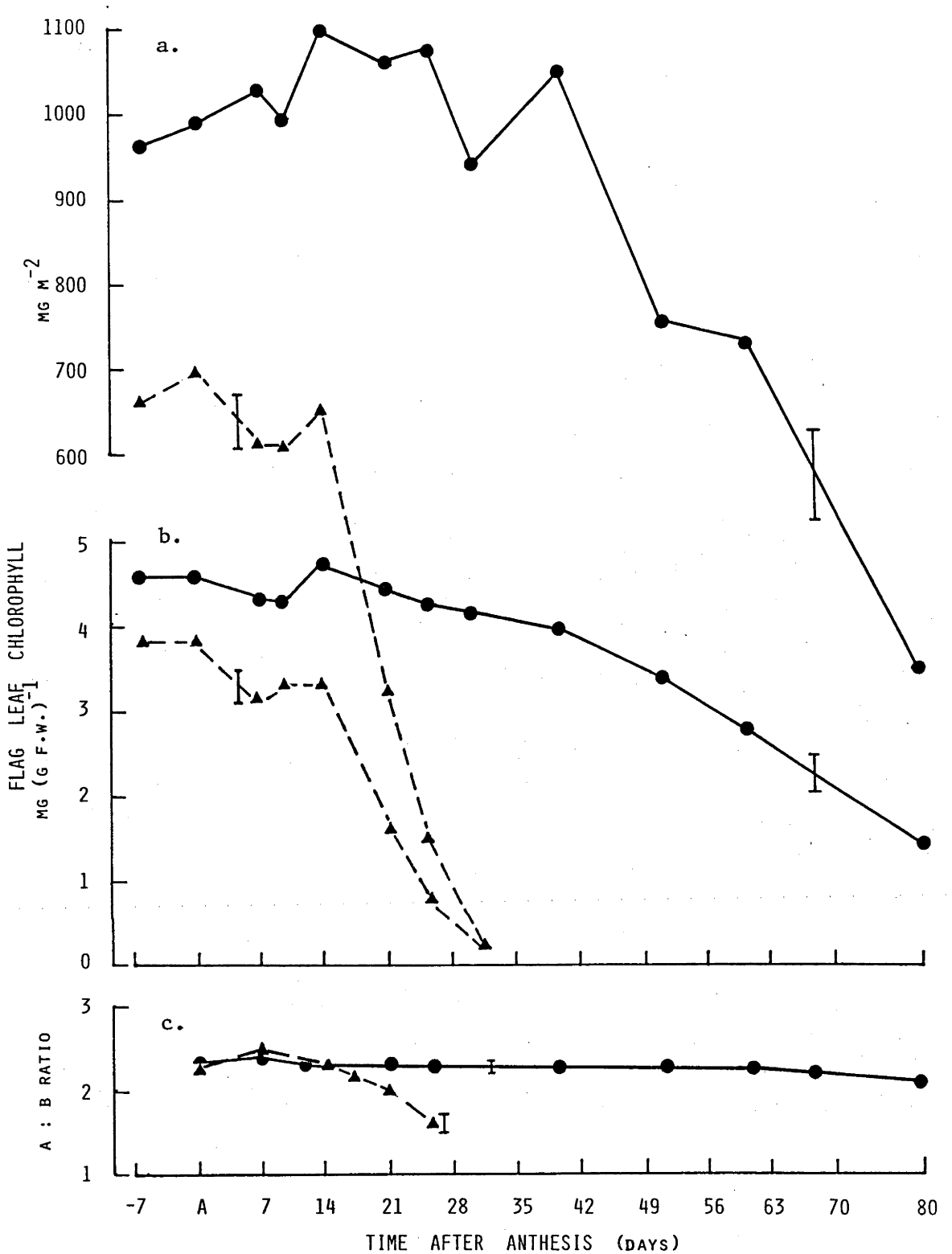


Figure 4.4 Chlorophyll in control (●—●) and low P (▲---▲) flag leaf tissue during the grain development stage expressed as total chlorophyll per unit area (a) and fresh weight (b) and the chlorophyll a/b ratio (c).
Each data point is the mean of three replicates.
Vertical bars indicate the 2x the average standard error of the means.

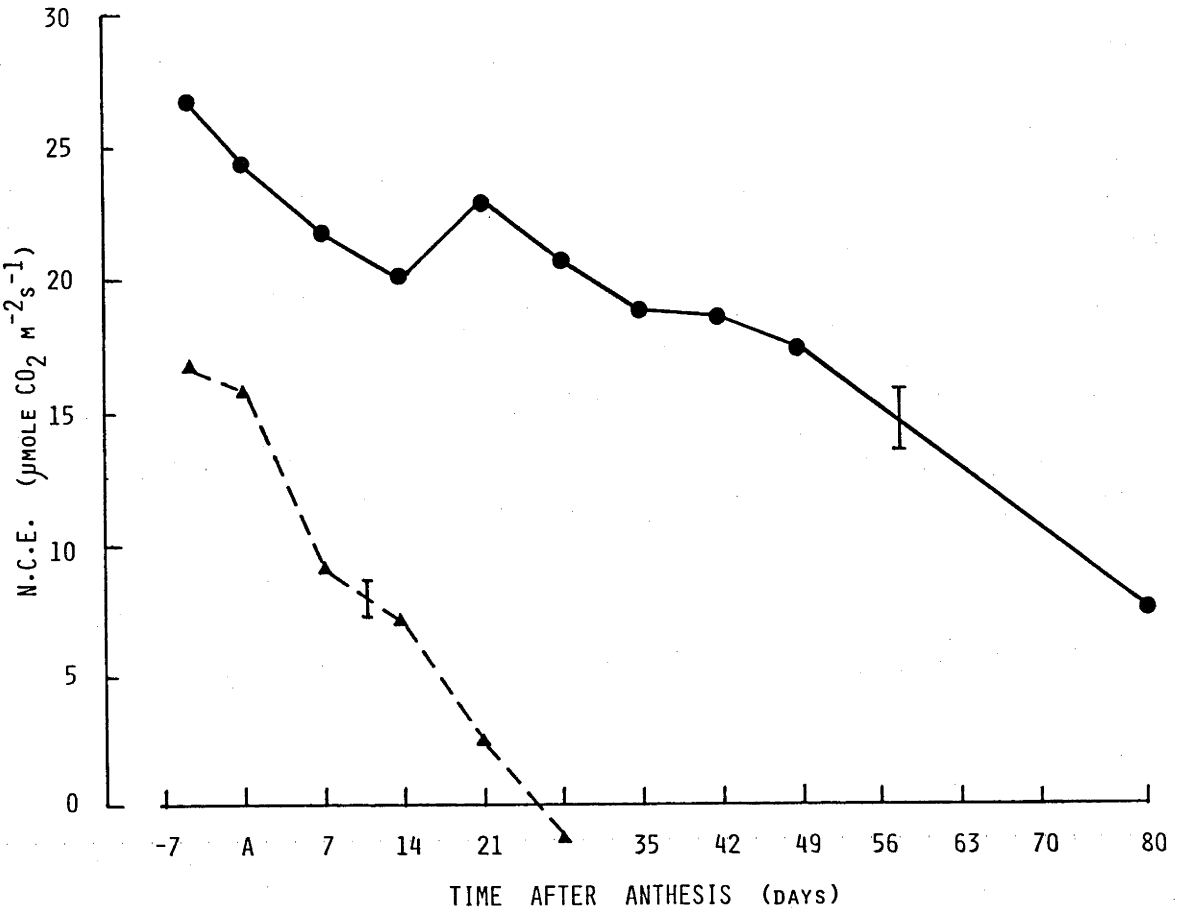


Figure 4.5 Photosynthesis (N.C.E.) of the flag leaf blade during the grain development stage in control (●—●) and low P (▲--▲) plants.
Each data point is the mean of three replicates.
Vertical bars indicate the 2x the average standard error of the means.

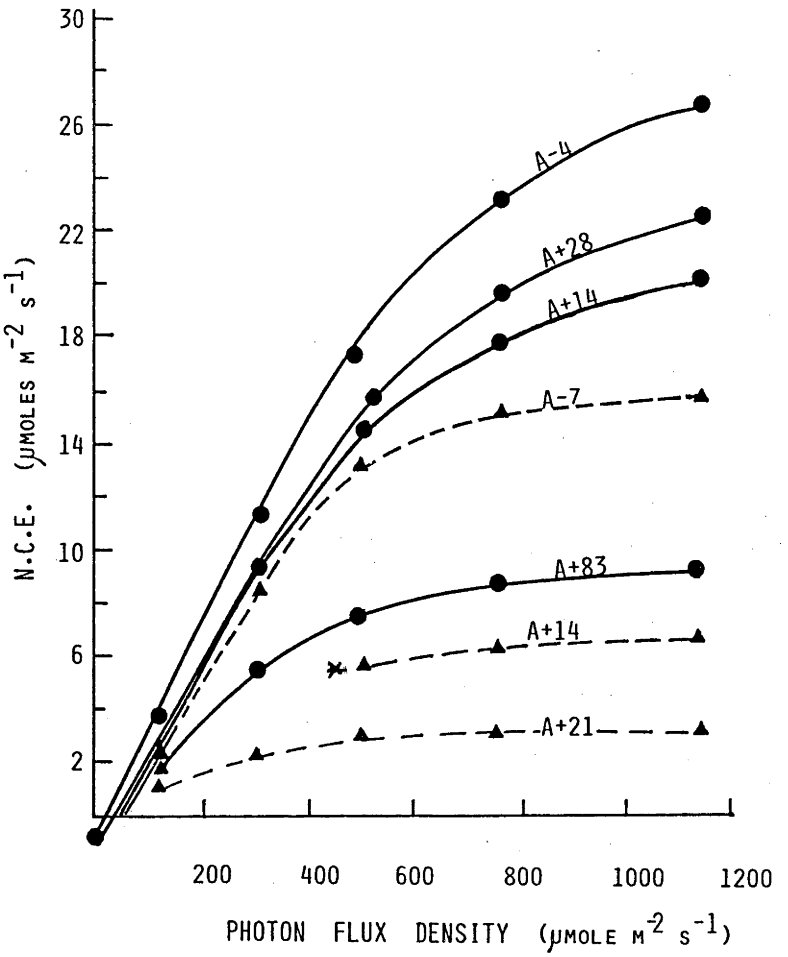


Figure 4.6 Light response curves for the flag leaf of control (●—●) and low P plants (▲--▲) during the grain development stage. A ± # indicates the number of days before and after anthesis. Each point is an average for two plants.

* equipment failure

Maximum N.C.E. in low P plants 7 days prior to anthesis was attained at 750 μmoles m^{-2} s^{-1}, and at even lower photon flux densities as the plants senesced. With senescence in both treatments there was a decline in the initial slope of the response to increasing light i.e. the quantum yield efficiency decreased with senescence.

4.3.2 Grain growth

The dry weight and water content of central spikelet grains are presented in Figures 4.7 and 4.8 respectively.

During the linear phase of grain filling (plotted using the method described by Sofield et al., 1977a) control plants accumulated dry matter at 1.46 mg $grain^{-1}$ day^{-1} compared to 1.12 mg $grain^{-1}$ day^{-1} for low P plants.

The maximum grain dry weight was not reached at a clearly defined time after anthesis in either treatment so six estimates are compared (Table 4.3). In each comparison the duration of grain filling was longer in control plants. These dry weight accumulation curves are discussed below in relation to grain phosphorus.

The lower grain dry weight accumulation rate and final grain size of low P plants were not accounted for by the 12% (not significant by Student's t-test) reduction in endosperm cell numbers. There were 66,500 (±3770) cells per control grain and 58,360 (±3910) cells per low P grain.

Both the absolute amount of water per grain and water as a proportion of the fresh weight were always greater for control than for low P grains (Figure 4.8).

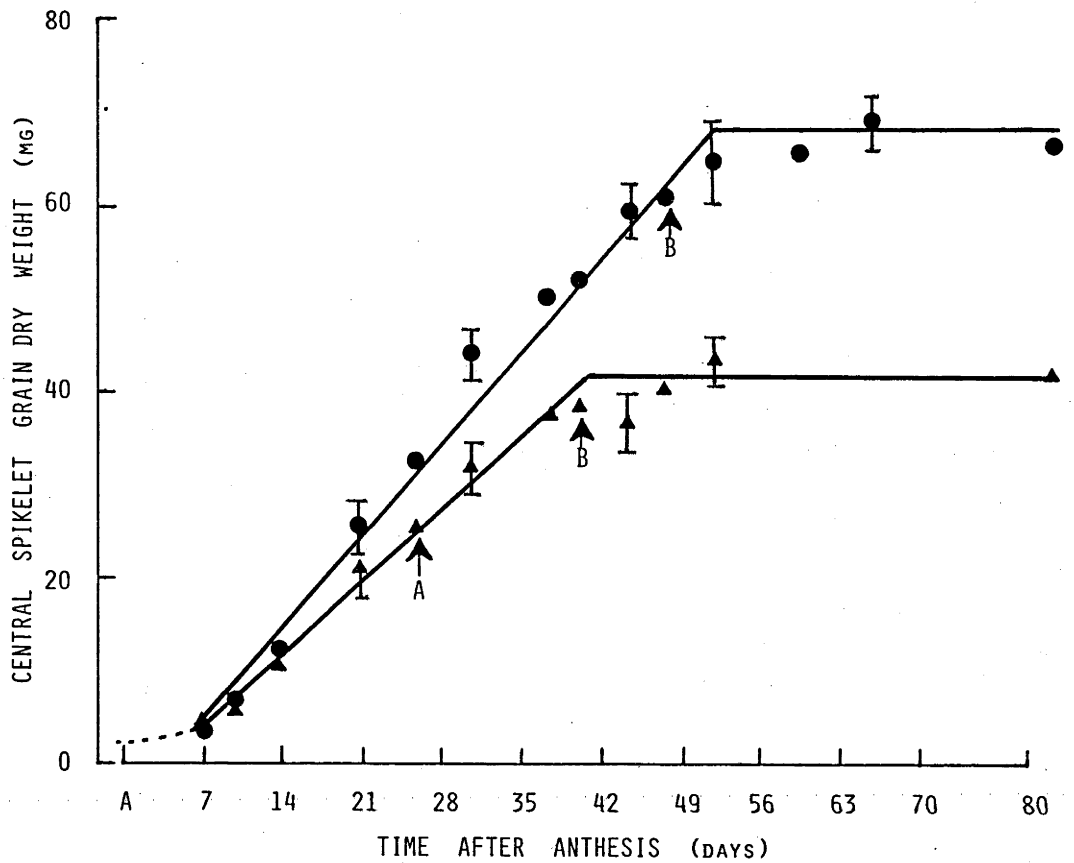


Figure 4.7 Dry weight accumulation in central spikelet grains of control (●—●) and low P plants (▲—▲).
The rates of dry weight accumulation in the linear phase were 1.46 mg day^{-1} (R^2 = 0.99, n = 11) for control and 1.12 (R^2 = 0.99; n = 8) for low P plants.
Arrow A = stage at which the low P flag leaf blade was 100% yellow; B = beginning of rapid loss of chlorophyll from the grain.

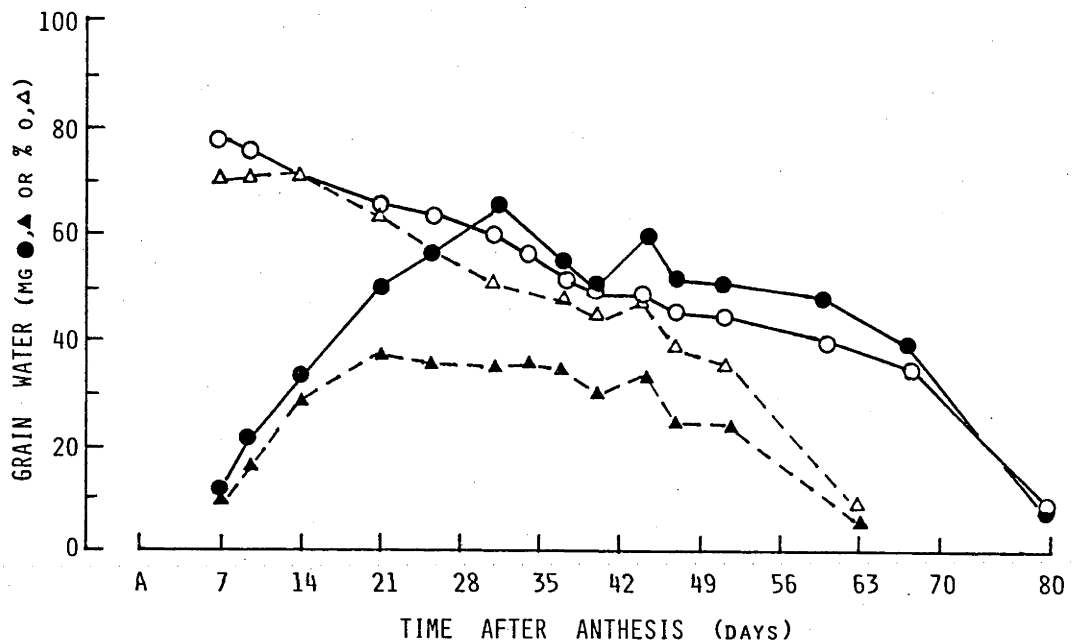


Figure 4.8 Water in central spikelet grains of control (——) and low P plants (---). Absolute amount (●,▲) and proportions of fresh weight (○,△).
Each data point is the mean of three replicates.

4.3.3 Plant phosphorus during grain filling

4.3.3.1 Flag leaf phosphorus

In the flag leaf of control plants the levels of ester, lipid and residue phosphorus compounds were maintained throughout the grain development phase. The inorganic and, as a result, the total phosphorus levels increased during the early stage of grain filling and remained high through to the final harvest (Figure 4.9a, 4.10a).

The total phosphorus concentration in the flag leaf of control plants fluctuated between 0.45 and 0.72% between 7 and 60 days after anthesis (Figure 4.9a). The reason for the anthesis level being slightly lower than this is not clear.

The flag leaf of the low P plants had only 0.11% P at anthesis and, from 7 days after anthesis, this level declined rapidly to 0.03% (Figure 4.9b - note change of scale compared to 4.9a).

In low P plants all phosphorus fractions declined during the first 30 days of grain filling (Figure 4.9b) and the ester phosphate pool was exhausted 26 days after anthesis. Relative to the total phosphorus lipid phosphate increased slightly whereas the inorganic and residue forms maintained proportions of 30 and 40% respectively (Figure 4.10b).

4.3.3.2 Grain phosphorus

The total amount of phosphorus per grain increased up to day 60 after anthesis in control plants and to day 40 in low P plants (Figure 4.11a). In the grain of control plants the total phosphorus which accumulated was predominantly in the form of phytate but inorganic +

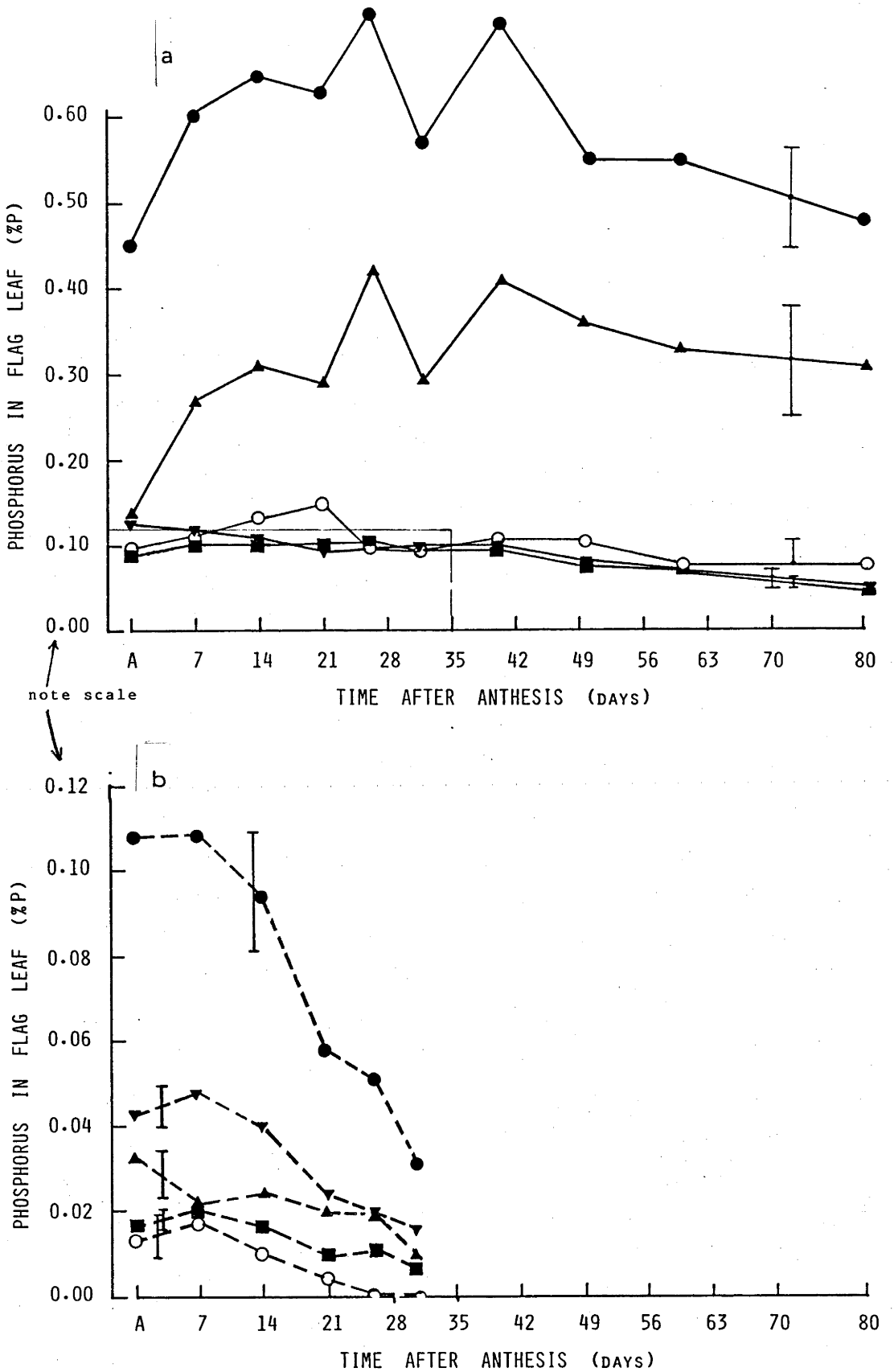
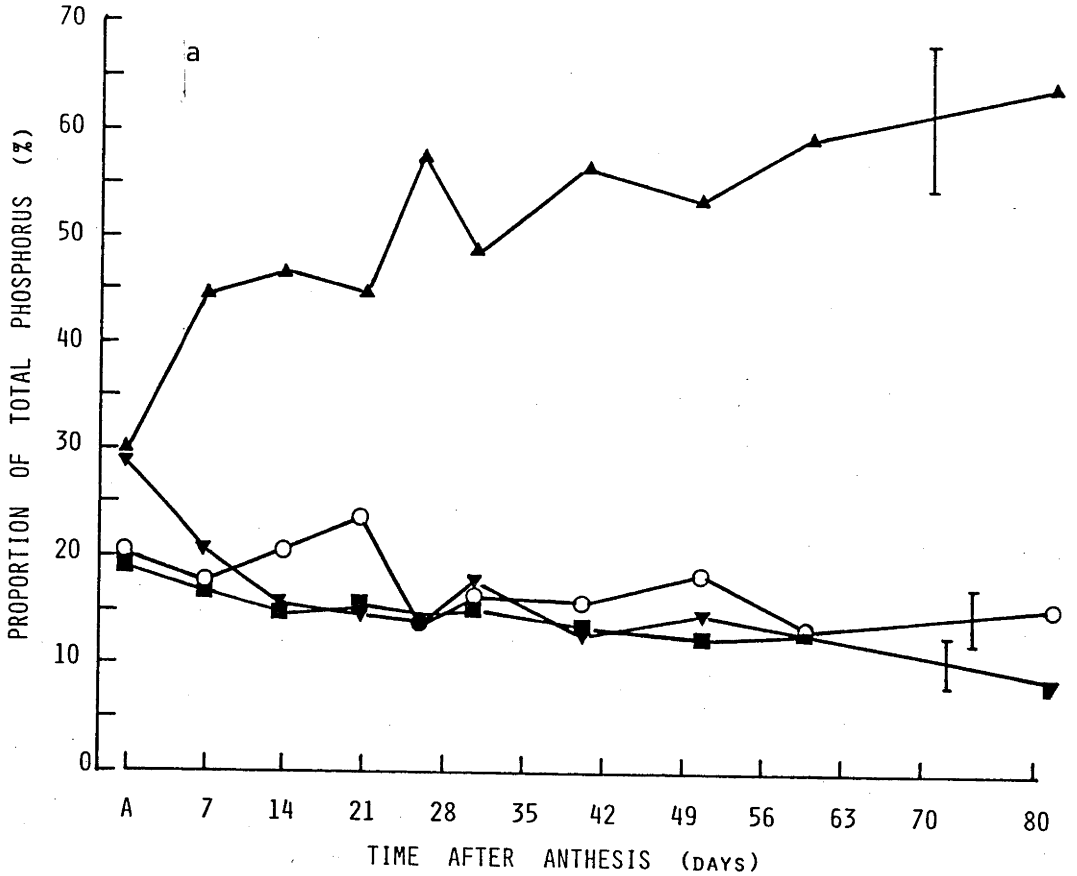


Figure 4.9 Forms of phosphorus (%P) in the flag leaf of control (a) and low P plants (b) during the grain development stage.
● total, ▲ inorganic, ■ lipid, ▼ residue, and O ester phosphorus.
Each data point is the mean of two replicates.
Vertical bars indicate the 2x the average standard error of the means.



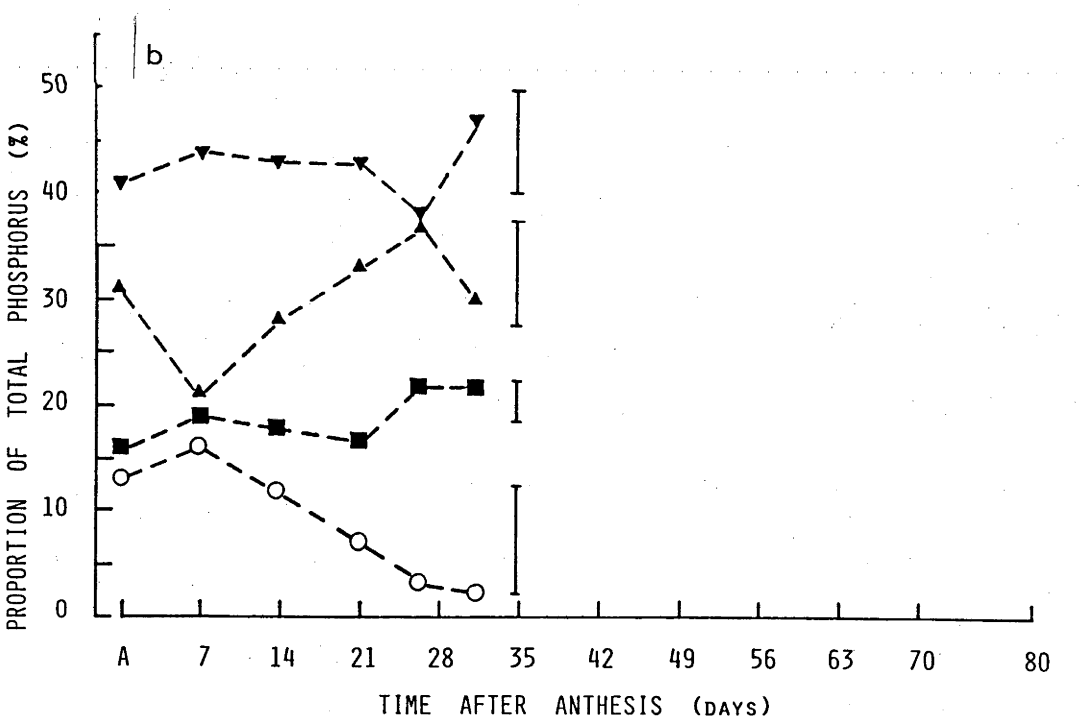


Figure 4.10 Phosphorus fractions in flag leaves expressed as a proportion (%) of the total phosphorus.
Symbols as in Figure 4.9.

ester phosphorus increased up to day 60 and then declined rapidly as the grain lost water.

The pattern of accumulation of phosphorus in grains of low P plants was quite different (Figure 4.11b). The inorganic + ester phosphorus rose to a maximum level 26 days after anthesis then declined slowly till maturity. Phytate phosphorus did not occur in significant amounts until 34 days after anthesis. By 40 days after anthesis phytate was the major form of phosphorus in the grains.

Residue phosphorus increased during the first 31 days of grain development in both control and low P grains.

The levels of phosphorus are presented as concentrations (% D.W.) in Figure 4.12. In grains of control plants total phosphorus fell initially then increased slightly towards day 60 and then declined slightly. Phytate phosphorus increased, while inorganic + ester and residue decreased, throughout grain filling.

In low P grains total phosphorus declined from 0.27% to 0.14% at day 31, then increased to 0.17%. The inorganic + ester and residue phosphorus concentrations declined throughout while the phytate phosphorus rose rapidly between 31 and 44 days after anthesis. This was not associated with a rapid decrease in grain water content (Figure 4.8).

The molar concentrations of inorganic + ester phosphorus were higher in control grains initially and rose during grain filling to 60 mM P at day 60. In low P grains the concentration fell during the early phase of grain filling before returning to 20 mM P. This concentration was maintained until maximum dry weight was attained. As the grains in either treatment ripened the molar concentrations of phosphorus declined. But at the final harvest, when grains were

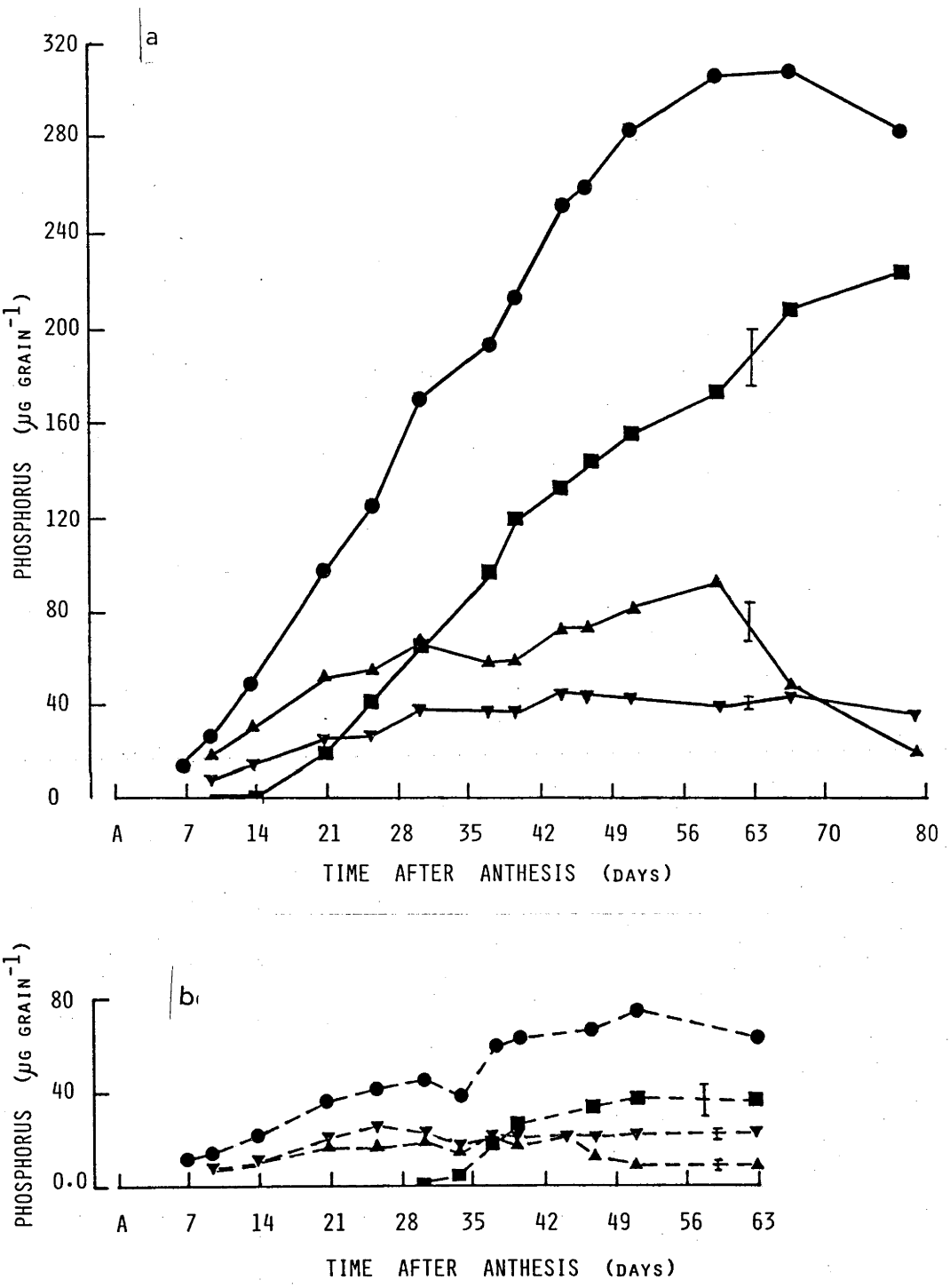
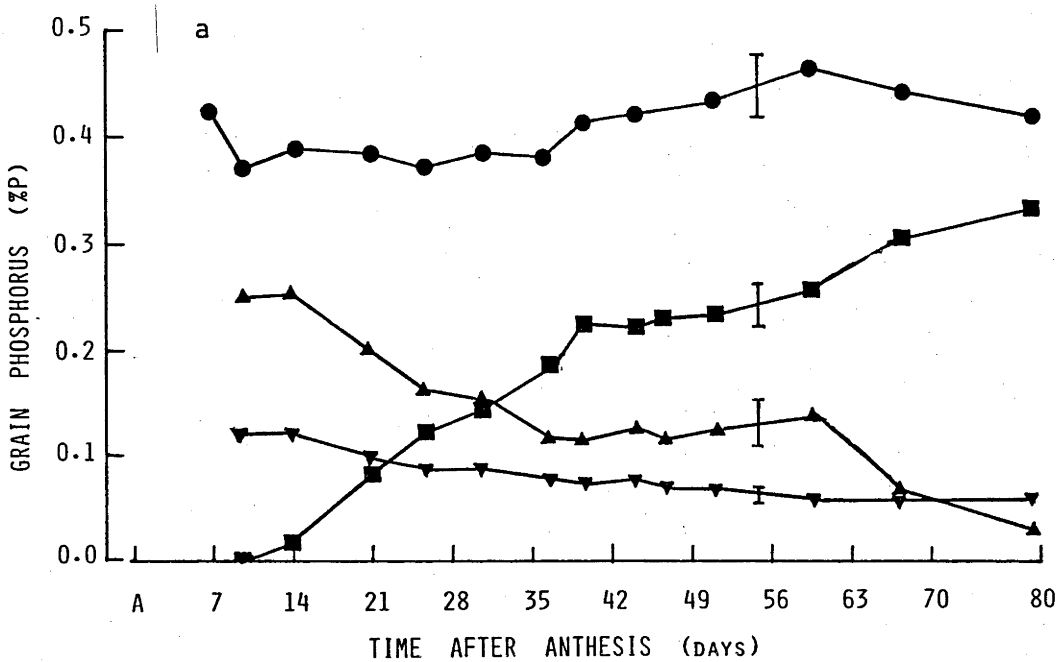


Figure 4.11 Accumulation of phosphorus (µg P) in developing wheat grains. a, control and b, low P plants.
● total, ■ phytate bound, ▲ inorganic plus ester, and ▼ residue phosphorus.
Each data point is the mean of two replicates.
Vertical bars indicate the 2x the average standard error of the means.



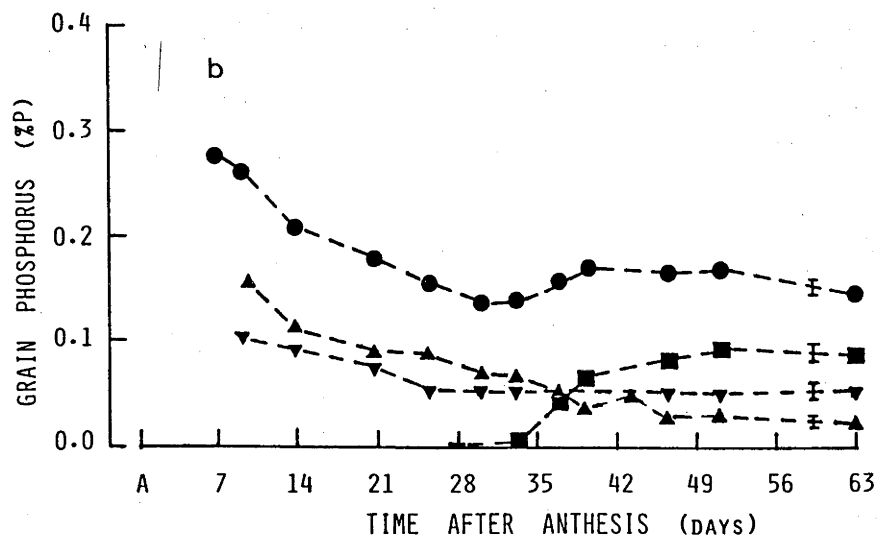


Figure 4.12 Phosphorus (% P) in developing wheat grains. Symbols as per Figure 4.11.

completely ripe, inorganic ester phosphorus was at the highest level recorded (Figure 4.13).

4.4 DISCUSSION

4.4.1 Grain development in relation to senescence

At maturity the dry weights of grains produced by plants on the low P regime were only 62% of those from control plants. This was due to a 23% reduction in the rate of accumulation of grain dry weight and a 22% reduction in the duration of grain filling. The lower rate of increase in grain dry weight may have been due to either the rate of supply of assimilate from the "source" tissues, or the rate of utilization of the available assimilate within the grain.

In low P plants the area of the flag leaf and the rate of assimilation of carbon per unit leaf area and the number of grains per ear were 62 to 65% of the control (Table 4.4). Both the area of photosynthetic tissue of the leaves and the rate of assimilation of carbon per unit leaf area declined rapidly so that the flag leaf blade, which may contribute 25% of the carbon deposited in the grain of plants on high levels of nutrition (Figure 1.5), was ineffective 28 days after anthesis (Figures 4.4, 4.5).

Despite these reductions in photosynthesis, with the complete senescence of the flag leaf blade, and the subsequent decline of photosynthesis in the flag leaf sheath, peduncle and the ear, there was not a significant change in the slope of grain dry weight accumulation until 90% of the maximum dry weight had been reached (Figure 4.7). Forty percent of the final grain dry weight was added after the flag leaf blade senesced. If all of the dry weight lost from

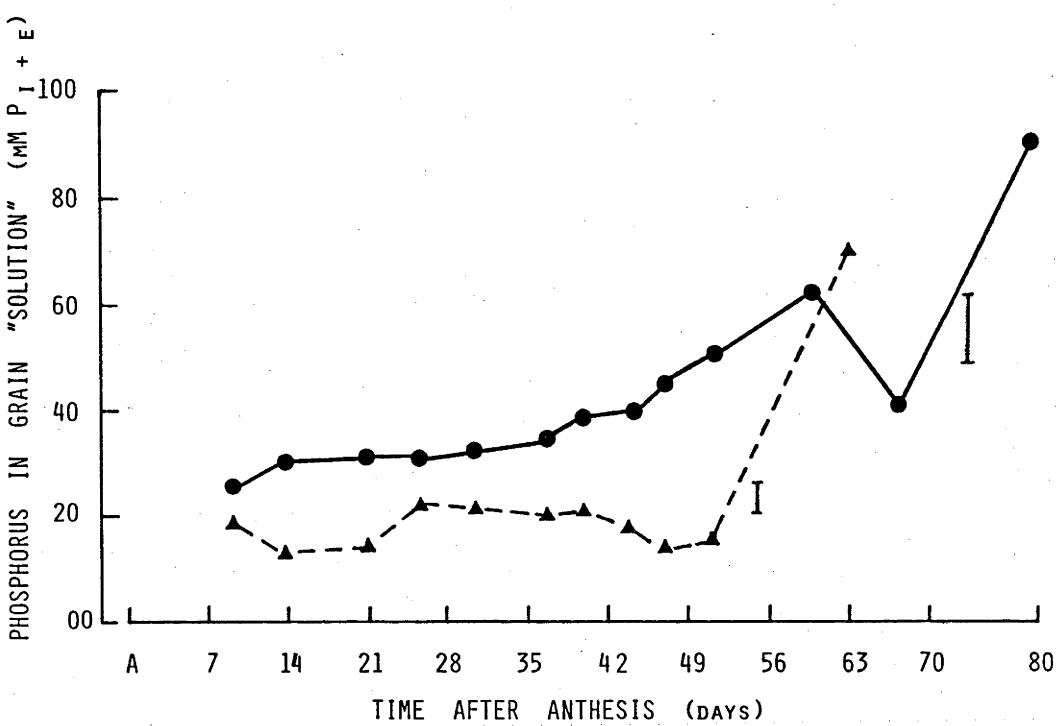


Figure 4.13 Concentration of inorganic + ester phosphorus in the grain "solution" (grain fresh weight-dry weight), in control (●) and low P (▲) plants.

Grain solution = water lost from grain on freeze drying

the stem (about 1.8 g from each of the internodes shown in Figure 4.2) was transferred to the grain this could account for, at best, 6 mg of dry weight per grain or 15% of the final dry weight. This suggests that the final 25% of the grain dry weight is derived from photosynthesis by the glumes, peduncle and flag leaf sheath.

If the days to 35% grain water (Table 4.3) more correctly describes grain filling under the low P regime then the final 10% of dry weight was achieved at the average rate of approximately 0.4 mg $grain^{-1}$ day^{-1}. In this period the grain lost the majority of its chlorophyll (Table 4.2).

In complete contrast to the low P plants, which senesced rapidly after anthesis, the control plants maintained active photosynthetic vegetative organs for longer than the grain filling period.

At the time the grains reached maximum dry weight the level of chlorophyll and the photosynthetic rate were similar to those of the low P plant at anthesis, but even 30 days later, at 82 days after anthesis, the flag leaf was still photosynthesizing. Much of the carbon assimilated at this stage is directed to the roots and young tillers (Chapter 5).

4.4.2 Senescence in relation to phosphorus

At anthesis the low P plants had been growing without phosphorus in the daily nutrient solution for 36 days. During this period the availability of phosphorus declined so that little phosphorus was taken up by the plant during grain filling (Chapter 3). At anthesis the flag leaf of the control plants contained four times as much total phosphorus as the low P plants and the concentrations of all the major forms of phosphorus were higher in the control leaves than in low P leaves. This is in agreement with

the suggestion of Bieleski (1968b) and Chapin and Bieleski (1982) that the organic compounds also have a 'storage' function.

In the course of senescence in low P plants inorganic and residue phosphorus accounted for the same proportions of the total phosphorus; lipid phosphorus increased slightly and ester phosphorus was depleted. Thus all forms of phosphorus were utilized as the leaf exported phosphorus (both the leaf specific dry weight and the concentration of phosphorus in the dry weight declined). If part of the inorganic phosphorus was "held" in the vacuole then it

ent utilization

Bieleski (1968b) stated that ... "When non-metabolic P_i (inorganic phosphorus) has disappeared growth ceases, for the remaining P_i cannot be utilized for growth". The "non-metabolic" pool is generally regarded as that in the vacuole. Exchange of phosphorus between the vacuole and cytoplasm is relatively slow - with a half life of 3 or more days (Bieleski, 1973). On the the other hand, the rate of turnover of ester compounds is rapid, especially under phosphorus deficiency (Bieleski, 1968) and as a result esters are vulnerable to degradation. Here leaf photosynthesis declined until either the ester phosphates, or the vacuolar pool, or both, were exhausted.

Phospholipid compounds make up roughly 25% of the lipids of the chloroplast envelope (Douce and Joyard, 1981) and 15% of the light harvesting chlorophyll protein complex (Selstam, 1981), as well as other key cell membrane structures (tonoplast, plasma membranes etc.). Simon (1974) argued that cell breakdown must occur if phospholipid is depleted during senescence and membranes are likely

to have gaps and allow solute leakage to take place. Nooden and Leopold (1978) suggested that the demise of a cell may follow the release of hydrolytic enzymes from the vacuole when the tonoplast membrane breaks down.

From his work with Spirodela plants Bieleski (1968a) concluded that the patterns of individual esters and lipids remained substantially unaltered when deprived of a supply of phosphorus. However he did show that the relative proportion of phosphatidyl glycerol fell from 13.9 to 6.8% under a minus-P treatment. This lipid was also found to be sensitive to water stress (Chetal et al., 1983) and senescence (Draper, 1969). Phosphatidyl glycerol forms about 15% of lipid in the light harvesting chlorophyll protein complex (Selstam, 1981) and is possibly a key compound in determining the ability of leaves to maintain photosynthesis under phosphorus depletion.

Camp et al. (1982) reported that senescing wheat leaves appear to lose whole chloroplasts, as opposed to a general degradation of all chloroplasts. They also noted a decline in the chlorophyll a/b ratio (as found here in Figure 4.4c) and suggested that this indicated changes in the chloroplast membranes.

It is concluded that depletion of lipid and exhaustion of ester phosphorus compounds, together with the possible exhaustion of metabolisable inorganic phosphorus, resulted in phosphorus induced senescence of the low P plants in this study.

4.4.3 Grain development in relation to phosphorus

The accumulation of phosphorus to day 60 in control plants and 49 in low P plants is evidence that the duration of grain filling was underestimated by several methods shown in Table 4.3.

Dodds (1957) found that windrowing wheat with more than 35% moisture in the grain resulted in decreased yields. Later Green et al. (1975) reported that the entry of water and phosphorus into grains of windrowed wheat ceased at this grain moisture level. Sofield et al. (1977b) also show maximum grain dry weight occurs at the 35% moisture level.

If this was also the case here (Figure 4.7) then the rate of increase in grain dry weight was depressed after 30 days. This may have been due to an excess of inorganic phosphorus in the endosperm of control grains.

In the present experiment the control plants maintained grain inorganic + ester phosphorus at 30 mM during the first 30 days of grain filling. Between days 30 and 60 the concentration doubled (Figure 4.13). This suggests that between days 30 and 60 the grain was unable to produce sufficient phytate to balance the intake of phosphorus. The physiological roles suggested for phytate were presented in Chapter 1.3.4.

If phytate only forms in the aleurone and scutellum cells then the failure to control the total inorganic + ester concentrations may have been due to a mass action effect whereby the phosphorus passed through the aleurone layer without adequate exposure to phytate precursors and/or phosphoinositol kinase (Figure 1.13). The relatively small increase in the rate of phytate formation between days 60 and 67 was associated with reductions in inorganic + ester phosphorus (Figure 4.13) and water content (Figure 4.8) of the grain as Jennings and Morton (1963) found.

Earlier workers found significant amounts of phytate in wheat grain as early as 10 days after anthesis (Williams, 1970; "potting

soil" in glasshouse; Nahapetian and Bassiri, 1975; field); and 20 days after anthesis (Jennings and Morton, 1963; field grown wheat; Michael et al., 1980; sandy soil with peat and 0.26 g P pot^{-1} at sowing). The low P plants here did not begin to accumulate phytate until 34 days after anthesis. Phytate rose between days 34 and 47. This coincided with the cessation of grain dry weight accumulation (Figure 4.7) but was not associated with definite reductions in inorganic + ester phosphorus or water content of the grain (Figures 4.11, 4.12 and 4.8). Perhaps phytate has a role in grain maturation in low P or field grown plants. Nahapetian and Bassiri (1975, 1976) and Bassiri and Nahapetian (1977, 1979) found some significant correlations between phytate and calcium and magnesium. In their 1975 study phytate formation coincided with chlorophyll destruction in the leaves which would release magnesium. This was not the case in Low P plants in the present study. The reason(s) for phytate formation is not yet understood. The elemental composition of the electron dense globoid crystals in the aleurone cells is reported in Chapter 7.

Table 4.1 Comparison of selected features of plants grown under two phosphorus regimes - means with standard errors in parentheses.

	Tillers plant^{-1}	Height (cm)		Anthesis (days from sowing)	Ear of main culm			
					Spikelets		Grains	Flag leaf area at anthesis (cm^2)
		Anthesis	Maturity		Fertile	Sterile		
Control:	2 (detillered)	54 (1.5)	64 (1.6)	56.8 (0.6)	17.0 (0.3)	0 -	54 (2)	27 (2)
Low P:	1.5 (0.3)	43 (0.7)	47 (1.0)	53.8 (0.4)	13.2 (0.4)	2.1 (0.3)	35 (1)	17 (2)

Table 4.2 Visual assessments of glumes and grains

Days after anthesis	Glumes		Grain	
	Control	Low P	Control	Low P
34		50% Chl* lost		Chl declining
37				
40		70% Chl lost		Yellow-green
44		glumes yellow	Chl declining	
47	50% Chl* lost	Peduncle, 50%	Yellow-green	Only a trace
	Central spikelet D & E	Chl lost		of Chl
	grain glumes yellow			
51	Central spikelets yellow	Peduncle yellow	Only a trace of Chl	Hard dough
				stage
60	Only a trace of Chl on		Hard dough stage	
	lower spikelet outer glumes			

* Chl = chlorophyll content by visual assessment.

Table 4.3 Duration of the grain filling period in Control and Low P plants (days)

	Control	Low P
Estimated from:		
linear plot[1]	51	40
logistic plot[2]		
90%	48	41
95%	54	48
grain color[3]	47	40
grain water[4]		
35%	67	49
40%	60	45
Maximum grain dry weight (mg)	65	40

1 Sofield et al. (1977a).

2 % of maximum dry weight Ymax, where $Y = \frac{Ymax}{1 + \exp -(b+kx)}$ and x = days after anthesis.

3 see Table 4.2

4 % moisture content; see Figure 4.9 (Green et al., 1975).

Table 4.4 Relative differences between Control and Low P plants

	Control (absolute units)	Low P (% of Control value)
Flag leaf		
Area (cm^2)	27	63
N.C.E. at anthesis ($\mu mole\ m^{-2}\ s^{-1}$)	26	62
Phosphorus (Total P as % DW at anthesis)	0.45	25
Grain		
Number in ear	54	65
Dry weight increase ($mg\ grain^{-1}\ day^{-1}$)	1.46	77
Duration of grain filling (days)	51	78
Mature weight (mg)	65	62
Phosphorus (Total P as % DW at maturity)	0.43	45

CHAPTER 5

REDISTRIBUTION OF PHOTOSYNTHATE AND PHOSPHORUS FROM THE FLAG LEAF OF WHEAT DURING GRAIN FILLING

5.1 INTRODUCTION

The flag leaf supplies a significant proportion of the assimilate translocated to the grain (Figure 1.5) and the area of the flag leaf can be significantly related to the yield per ear (Rawson et al., 1983). However, under a declining phosphorus supply the flag leaf senesces during early grain development (Chapter 4). Studies are required to show the redistribution of phosphorus and carbon from such a leaf.

Zabluda and Prosteva (1956) reported that the movement of ^{32}P between tillers of a barley plant was greater at the tillering stage than at heading. They found less movement of ^{32}P from subsidiary tillers to the main culm than from the main to the subsidiary tillers.

Marshall and Wardlaw (1973) studied the movement of carbon and phosphorus in the main culm of wheat during mid grain filling. Their work suggested that the movement of phosphorus from leaves was largely determined by the movement and demand for carbohydrate within the plant and not by a phosphorus requirement of the "sink". However, in plants with a reduced supply of phosphorus slightly more ^{32}P moved towards the roots and tillers than in a control plant (8.1% compared to 4.3%). However compared to the treatments reported in Chapters 3 and 4 of this thesis, the low phosphorus plant in that study was probably only slightly different to the control.

Three experiments are reported here.

Experiment 1 examined the redistribution of phosphorus from the flag leaf of wheat grown with a range of phosphorus regimes;

Experiment 2 examined the redistribution of phosphorus and carbon from the flag leaf at early, mid and late grain filling, and

Experiment 3 examined the deposition of phosphorus exported from the flag leaf in stems and grain.

5.2 MATERIALS AND METHODS

5.2.1 General

Wheat (cv. Kite) plants were grown as described in Chapter 2 in a glasshouse controlled to 18° by day and 13°C at night.

Plants were labelled and the redistribution of label assessed by methods described in Chapter 2.8.

5.2.2 Experiment 1

Plants were grown with daily applications of nutrient solution which contained 0.25 mM P for 15 days, or 1 mM P until floral initiation (23 days after sowing), tillering (33 days), flag leaf emergence (43 days), anthesis (64 days), or mid grain filling (85 days = day assessed). At each of these growth stages four plants were transferred to nutrient solution which contained no phosphorus.

Twenty days after the ear of the main culm anthesed the flag leaf was labelled with 74×10^3 Bq of ^{32}P. The plants were harvested 24 hours later. The redistribution of phosphorus in the main culm, root and crown was measured. Other tillers were discarded.

5.2.3 Experiment 2

Plants were grown at two phosphorus regimes; either 1 mM P daily throughout the experiment (control) or 0.25 mM P daily for 18 days and then a nil P solution (low P). Control plants were detillered regularly.

At 14, 26 and 40 days after anthesis six plants were taken from each treatment and labelled with both ^{14}C and ^{32}P (74×10^3 Bq).

The plants were harvested 24 hours later and the redistribution of both carbon and phosphorus was measured.

5.2.4 Experiment 3

Control and low P plants were grown as just described. At 26 days after anthesis four plants were labelled with 185 x 10^3 Bq ^{32}P applied to the flag leaf blade. The redistribution of phosphorus was determined as before. The phosphorus in stems and grains was fractionated as described in Chapter 2.6 (^{31}P) and 2.8 (^{32}P).

Statistics

The redistribution of phosphorus or carbon throughout the plant was interpreted with the aid of Principal Components Analysis (PCA; Hope, 1968; Williams, 1976) using the Genstat Stastistical Package (Lawes Agricultural Trust, 1980). PCA is not scale invariant, i.e. variables with a large variance will dominate the analysis, so analyses were performed on standardized data (i.e. mean zero with unit standard deviation). The PCA and correlation analyses were performed without considering the treatment structures as it was not considered necessary to examine independently each line of the analysis of variance.

5.3 RESULTS

5.3.1 Experiment 1

Data for the distribution of dry weight and ^{32}P exported from the flag leaf are presented in Table 5.1 and 5.2. The means and standard errors are for analysis of untransformed data.

The number of tillers per plants increased with the phosphorus supply. Only a main culm was produced by plants grown on the Low

P regime, while 11 tillers formed on control plants. The additional tillers reduced the dry weight of the main culm (including root and crown), possibly due to shading.

The grains at the time of sampling were in the early phase of linear dry weight accumulation (Figure 4.7) and only made up 14.6 to 20.8% of the shoot dry weight. However grains accumulated from 30 to 80% of the phosphorus which was exported from the flag leaf blade in 24 hours (Table 5.2). In plants supplied with phosphorus only during the early growing period the major proportion of exported phosphorus was in stems and the flag leaf sheath. In Control plants 80% of the exported phosphorus was in the grain and only a small amount was found in the stem.

The correlation matrix (Table 5.3) shows that the distribution of phosphorus to grain was highly negatively correlated with that in the stem, flag leaf sheath and root/crown.

Principal component analysis of standardized data showed that the first three eigen values (values 3.2, 1.6 and 1) explained 82.4% of the total variance (Table 5.4). The first principal component identified grain as the dominant phosphorus accumulating tissue in contrast with the lower stem and root/crown. The second principal component suggested that chaff, lower leaves and root/crown accumulated phosphorus in contrast with the flag leaf sheath. The third principal component identified a contrast between the peduncle and the tissues connected to it (chaff and flag leaf sheath).

5.2.3 Experiment 2

The proportions of exported phosphorus (^{32}P) and carbon ($^{14}CO_2$) in each tissue of the plant are presented in Tables 5.5, and 5.6 and

their ratio in Table 5.7 respectively. By 40 days after anthesis the flag leaves of all low P plants were dead and could not be labelled.

The grain attracted more carbon and phosphorus in the 24 hours following labelling on day 26 than at day 14 or day 40. At day 14 peduncle and lower stem contained significant amounts of the exported carbon. The distribution of phosphorus was similar to that for carbon in control plants. Low P plants had significant amounts of phosphorus in chaff and the flag leaf sheath. At day 40 the control plants directed 56 and 38% of the exported carbon and phosphorus respectively to the root, crown and tillers.

Deposition of carbon into the grain, relative to phosphorus, was higher in low P plants than in the control and declined with age in both treatments.

Correlation matrices for the distribution of carbon and phosphorus exported from the flag leaf blade over 24 hours are presented in Table 5.8. The proportion of exported carbon in the grain was negatively correlated with that in the root/crown/tillers. The amounts in the flag leaf sheath, peduncle and lower stem were highly correlated.

The proportion of exported phosphorus in the grain was negatively correlated with that in all other tissues. The phosphorus which accumulated in the root/crown/tillers showed significant negative correlations with that in the chaff and flag leaf sheath. Significant positive correlations occurred between chaff and other shoot parts and between peduncle, leaf sheath, lower stem and lower leaf.

Tables 5.9 and 5.10 contain the principal component analyses of the distribution of exported carbon and phosphorus.

Carbon

The first principal component indicated a contrast of stem (peduncle and lower) and flag leaf sheath with root/crown/tillers. The second principal component showed a contrast of the ear with the root/crown/tillers. The third principal component was a contrast of the grain with chaff, lower leaves and root/crown/tillers.

Phosphorus

Two principal components explained 75.6% of the variation. The first revealed a contrast of grain and root/crown/tillers with all other tissues; the second a contrast of ear and flag leaf sheath with lower stem and root/crown/tillers.

5.3.3 Experiment 3

5.3.3.1 Distribution of phosphorus exported from the flag leaf blade

Table 5.11 shows the distribution of the phosphorus exported from the flag leaf blade over 24 and 168 hours after labelling with ^{32}P.

After 24 hours 58% of the exported phosphorus was in the grain in control plants compared to 44% in low P plants. These proportions are both lower than for the comparable sampling in experiment 2. Here more phosphorus was directed to the lower stem (especially in low P plants) and more to the root/crown/tillers.

In control plants the distribution of exported phosphorus did not change markedly between 24 hours and 168 hours after the ^{32}P was applied. In low P plants the grains had accumulated 75.6% of the exported phosphorus at 168 hours, compared to 61.6% in grain of control plants. The proportion in the flag leaf sheath, lower leaves

and root/crown/tillers was similar, while the proportions in chaff, peduncle and lower stem decreased between 24 and 168 hours.

The correlation matrix and principal component analysis of these data are not presented but confirmed the relationships established in experiments 1 and 2. There were strong negative correlations between the proportions of phosphorus in grain and the rest of the plant, especially stem tissues; and strong positive correlations between chaff, stem and flag leaf sheath. The first two principal components, which accounted for 85% of the variation, followed the patterns shown in Table 5.10.

5.3.3.2 Fractionation of phosphorus deposited in stems and grain

Table 5.12 contains the analysis of stem phosphorus. In control plants inorganic and lipid phosphorus made up 91% of the total amount of ^{31}P phosphorus at both harvests. In low P plants only 2.5% of the phosphorus was inorganic and 66% was in the lipid fraction. In both treatments the absolute amount of phosphorus in each of the four fractions declined slightly between 24 and 168 hours after labelling, and the proportions of phosphorus were maintained.

At 24 hours after labelling 55 and 53% of the ^{32}P was in the inorganic form in control and low P plants respectively. Control plants had a higher proportion of ^{32}P in the ester pool, but a lower proportion in the residue pool than did the low P plants. Additional ^{32}P accumulated in the stems of control plants between 24 and 168 hours after labelling and there was a slight shift of ^{32}P from the ester to the inorganic pool in absolute (cpm) and relative terms (Table 5.12). In low P stems the absolute and relative amounts of

^{32}P in the ester and inorganic pools declined as ^{32}P in the lipid and residue pools increased. The specific activities of each pool (Table 5.13) clearly show that the small inorganic phosphorus pool in low P plants was the most actively labelled pool. Lipid compounds showed the lowest specific activity. In low P plants the specific activity of esters fell to less than half while that of lipids and residue more than doubled between 24 and 168 hours after labelling.

Table 5.14 contains the analysis of grain phosphorus. The control grains had a much higher concentration of total phosphorus with a higher proportion in the phytate form. Between the 24 and 168 hour harvests the proportion of phytate increased in both treatments as inorganic + ester declined.

At 24 hours after labelling 89% of the ^{32}P was in the inorganic + ester pool (both treatments) but the proportion fell with time. In control grains ^{32}P accumulated in the phytate and to a lesser extent the residue fractions. In low P grains both phytate and residue held about 20% of the ^{32}P 168 hours after labelling. These changes are also shown by the specific activities of each fraction (Table 5.15). Only inorganic + ester and phytate (in control plants at 168 hours) had a specific activity greater than 1.0.

5.4 DISCUSSION

These studies extend the work of Zabluda and Prosteva (1956) and Marshall and Wardlaw (1973). Experiment 1 showed that wheat deposits less of the phosphorus exported from the flag leaf into grain and more into the stems when grown with a reduced supply of phosphorus; Experiments 2 and 3 that the distribution also varies with the stage of grain filling and the length of the redistribution period.

The patterns of distribution of carbon and phosphorus were broadly similar and apparently the movement of phosphorus followed the movement and demand for carbohydrate as Marshall and Wardlaw (1973) found. However, there were some significant departures from the general pattern and these indicate that translocation of carbon and phosphorus are not interdependent.

Low P plants retained little labelled carbon but significant labelled phosphorus in the flag leaf. Low P plants also deposit more carbon than phosphorus into the grain especially during the early stage of grain development. This is in line with other work which shows that the concentration of phosphorus in grain declines, during the early stages of grain development but may increase during late grain filling (Sofield et al., 1977b; Figure 4.12). At maturity the grain of wheat contains up to 50% of the dry matter in the shoots (Donald and Hamblin, 1976; Austin, 1980) and 45 to 90% of the phosphorus (Lipsett, 1964; Waldren and Flowerday, 1979; Jessop et al., 1984; Batten and Khan, unpublished data). Low P plants have a lower harvest index than control plants (Table 3.3; Figure 7.1). If more assimilate stored in stems prior to anthesis was available for translocation to the grains this could both raise the harvest index and reduce the grain phosphorus concentration; because this stored assimilate is presumably associated with very low levels of phosphorus (Figure 3.1).

Cultivars with a higher yield or higher harvest index generally have a lower concentration of phosphorus in the grain (Lipsett, 1964, 1969; Batten and Khan, unpublished data; Chapter 7). Further studies are warranted on the translocation of phosphorus relative to currently assimilated carbon and pre-anthesis stored assimilates in a

range of genotypes, especially in field and low phosphorus situations. It is possible that this will reveal genotypes which retain phosphorus in photosynthetically active flag leaf tissue, thereby extending the duration of the flag leaf and producing more grain with a lower concentration of total and phytate phosphorus.

Does the stem use or store phosphorus retranslocated from the flag leaf during grain development? The stem (lower stem, peduncle and chaff) and root/crown/tillers (according to principal component analyses using normalized data) are also temporary stores of phosphorus (and carbon).

Fractionation of the phosphorus in the stem suggests that remobilized phosphorus initially enters the small inorganic pool and to a lesser extent the ester and other pools. With time the lipid and residue pools took up more labelled phosphorus, but the specific activity of the inorganic pool remained high and that of the esters declined. These changes suggest that the stem is simply a temporary store for phosphorus exported from the flag leaf until required by the developing grain.

The growth of low P grains was not reduced by a lack of available phosphorus within the grain (Chapter 6). Because only a small proportion of phytate was formed in low P grain, it is concluded that the short term build-up of retranslocated phosphorus in stems, occurs because phosphorus is exported from the flag leaf at a faster rate than it can be absorbed by the grain. In view of the senescence which follows the decline in leaf phosphorus, this is clearly an example of inefficient utilization of phosphorus by the wheat plant.

TABLE 5.1 Distribution of dry matter in plants grown with six phosphor regimes (experiment 1)

Phosphorus regime	1	2	3	4	5	6	
Time phosphorus supplied (days)	15 (Low P)[1]	23	33	43	64	85 (Control)	s.e.[2]
Tillers per plant at day 85	1	4	4	5	10	11	0.3
Dry weight of main tiller including crown (g)	3.75	4.31	4.67	4.53	4.22	4.20	0.2
Distribution of dry matter (%) total)							
grain	14.6	15.4	15.1	18.0	20.8	19.2	1.5
chaff[3]	14.3	13.7	11.1	11.7	11.5	11.6	0.7
peduncle	10.2	11.9	12.5	9.3	7.2	7.4	0.9
flag leaf sheath	6.0	5.1	4.0	5.2	5.6	5.6	0.6
lower stem	24.6	18.1	14.0	19.7	18.8	18.1	1.5
lower leaves	9.9[4]	7.3[4]	8.8[5]	10.4	12.2	12.0	1.0
root/crown	20.4	28.3	34.7	25.8	24.0	26.3	3.8
Grain yield components							
grain number	38	39	36	53	50	50	2.5
grain weight (mg)	14	17	20	15	18	16	1.0

[1] regimes 2-6 received 1 mM P solution; regime1received 0.25 mM P solution

[2] standard error of mean; data not transformed

[3] includes glumes, rachis and rachilla

[4] all leaves below flag were yellow

[5] lower leaves yellow

TABLE 5.2 Distribution of phosphorus (^{32}P) exported from the flag leaf blade in 24 hours in plants grown with six phosphorus regimes (experiment 1)

Phosphorus regime	1	2	3	4	5	6	s.e.
Time phosphorus supplied (days)	15 (low P)	23	33	43	64	85[1] (control)	
Distribution of labelled phosphorus (% total)[1]							
grain	31.0	29.8	30.0	43.5	68.5	80.3	5.2
chaff	8.0	8.0	4.5	3.8	4.3	2.5	1.8
peduncle	17.8	15.0	22.3	14.0	5.5	4.3	6.0
flag leaf sheath	21.8	20.8	17.0	24.5	14.5	5.8	4.1
lower stem	14.8	22.8	23.0	9.8	5.5	4.8	3.2
lower leaves	2.0	0.2	0.2	2.4	0.6	0.6	1.1
root/crown	5.0	3.8	3.8	1.5	1.5	1.5	1.1
Total relative counts	1617	1370	1408	676	884	1100	625

[1] ^{32}P was applied to plants 21 days after anthesis

TABLE 5.3 Correlation matrix of distribution of retranslocated phosphorus in wheat plants in experiment 1 (d.f. = 22; 0.404 = P < 0.05; 0.515 = P < 0.01)

1	grain	1.00						
2	chaff	-0.46	1.00					
3	flag leaf sheath	-0.63	0.15	1.00				
4	peduncle	-0.69	0.01	0.17	1.00			
5	lower stem	-0.82	0.44	0.39	0.36	1.00		
6	lower leaves	-0.03	0.33	-0.31	0.05	-0.13	1.00	
7	root/crown	-0.56	0.50	0.04	0.27	0.62	0.35	1.00
		1	2	3	4	5	6	7

TABLE 5.4 Principal components analysis of distribution of phosphorus (^{32}P) exported from the flag leaf blade in 24 hours in plants grown at six phosphorus regimes (experiment 1). The eigen vectors are given for each component

	Principal component				
	1	2	3	4	5
Plant tissue					
grain	+0.54	+0.14	+0.11	-0.15	+0.01
chaff	-0.33	+0.37	+0.47	+0.21	-0.65
peduncle	-0.33	-0.14	-0.77	+0.10	-0.33
flag leaf sheath	-0.29	-0.48	+0.31	+0.56	+0.38
lower stem	-0.49	-0.10	+0.15	-0.43	+0.02
lower leaves	-0.06	+0.67	-0.21	+0.51	+0.31
root/crown	-0.41	+0.37	+0.01	-0.41	+0.47
Eigen value	3.19	1.61	0.97	0.64	0.39
% of variation	45.6	23.0	13.8	9.2	5.6

TABLE 5.5 Distribution of carbon exported from the flag leaf of plants grown with contrasting phosphorus regimes; experiment 2
(% of total exported in 24 hours after 5 min labelling period)

Time after anthesis (days)	14		26		40	
Phosphorus regime	Control	Low P	Control	Low P	Control	s.e.
grain	42.3	53.6	71.9	57.7	31.2	4.0
chaff	6.6	11.3	6.0	12.6	3.0	1.8
peduncle	17.4	10.6	3.3	1.7	2.0	1.2
flag leaf sheath	1.3	1.9	0.6	0.7	0.6	0.2
lower stem	20.5	14.8	5.8	5.2	6.5	1.6
lower leaves	0.4	1.0	0.2	4.1	0.5	0.8
root/crown/tillers	11.3	6.9	12.4	17.8	56.0	3.8
Total relative counts	6487	1111	7103	237	4952	750

TABLE 5.6 Distribution of phosphorus exported from the flag leaf of plants grown with contrasting phosphorus regimes; experiment 2.
(% of total exported during 24 hours)

	14		26		40	
	Control	Low P	Control	Low P	Control	s.e.
grain	40.8	32.8	73.7	56.6	37.5	3.5
chaff	8.1	14.7	5.5	9.2	3.5	1.5
peduncle	12.5	15.9	5.0	8.1	5.4	1.3
flag leaf sheath	3.8	15.2	2.8	14.6	4.3	0.8
lower stem	17.3	16.5	6.8	9.6	10.3	2.0
lower leaves	0.5	3.4	0.3	0.8	1.1	1.2
root/crown/tillers	16.9	2.0	5.7	1.1	37.9	3.3
Total relative counts	2781	897	1439	1415	2156	450

TABLE 5.7 Ratio of proportion of exported carbon to proportion of exported phosphorus deposited in each plant part (experiment 2)

	14		26		40	
	Control	Low P	Control	Low P	Control	s.e.
grain	1.04	1.65	0.98	1.03	0.85	0.09
chaff	0.89	0.77	1.29	1.30	0.91	0.20
peduncle	1.42	0.67	0.61	0.22	0.41	0.11
flag leaf sheath	0.36	0.12	0.22	0.05	0.15	0.03
lower stem	1.20	0.91	0.83	0.64	0.66	0.12
lower leaves	1.10	1.30	0.80	33.0[1]	0.60	9.0[1]
root/crown/tillers	0.64	3.78	4.10	30.3[1]	1.5	7.1[1]
Total relative counts	2.55	1.42	5.41	0.27	2.66	0.67

[1]Ratio and s.e. not reliable because of the very low proportion of counts in these tissues.

TABLE 5.8 Correlation matrix of distribution of translocated carbon and phosphorus in wheat plants (experiment 2)
(d.f. = 27; 0.367 = P<0.05; 0.470 = P<0.01)

Carbon

1	grain	1.00					
2	chaff	0.25	1.00				
3	flag leaf sheath	-0.10	0.14	1.00			
4	peduncle	-0.22	0.05	0.67	1.00		
5	lower stem	-0.32	0.08	0.63	0.92	1.00	
6	lower leaves	0.01	0.70	-0.25	-0.27	-0.20	1.00
7	root/crown/tillers	-0.67	-0.55	-0.40	-0.49	-0.41	-0.10	1.00
		1	2	3	4	5	6	7

Phosphorus

1	grain	1.00					
2	chaff	-0.31	1.00				
3	flag leaf sheath	-0.28	0.69	1.00			
4	peduncle	-0.62	0.69	0.51	1.00		
5	lower stem	-0.70	0.44	0.21	0.73	1.00	
6	lower leaves	-0.41	0.38	0.32	0.45	0.27	1.00
7	root/crown/tillers	-0.40	-0.65	-0.55	-0.35	-0.07	-0.13	1.00
		1	2	3	4	5	6	7

TABLE 5.9 Principal component analysis of the distribution of carbon (^{14}C) exported from the flaf leaf blade in 24 hours in control and low P plants at three times during grain development (experiment 2). The eigen vectors are given for each component.

	Principal Component				
	1	2	3	4	5
Plant tissue					
grain	+0.03	+0.46	-0.65	+0.02	+0.18
chaff	-0.13	+0.57	+0.35	-0.16	-0.69
peduncle	-0.55	-0.12	+0.06	+0.31	+0.09
flag leaf sheath	-0.48	-0.06	+0.00	-0.84	+0.24
lower stem	-0.53	-0.14	+0.18	+0.36	-0.02
lower leaves	+0.13	+0.46	+0.58	+0.07	+0.65
root/crown/tillers	+0.38	-0.46	+0.30	-0.19	-0.08
Eigen values	+2.90	+2.16	+1.27	+0.44	+0.15
% of variation	41.4	30.9	18.2	+6.3	2.2

TABLE 5.10 Principal component analysis of the distribution of phosphorus (^{32}P) exported from the flag leaf blade in 24 hours in control and low P plants at three times during grain development (experiment 2). The eigen vectors are given for each component.

	Principal component				
	1	2	3	4	5
Plant tissue					
grain	0.33	0.55	0.02	-0.35	-0.14
chaff	-0.46	0.26	0.07	0.04	0.77
peduncle	-0.48	-0.09	0.18	-0.19	-0.01
flag leaf sheath	-0.38	0.30	-0.13	0.71	-0.43
lower stem	-0.38	-0.33	0.45	-0.33	-0.38
lower leaves	-0.31	-0.11	-0.86	-0.37	-0.11
root/crown/tillers	0.24	-0.64	-0.12	0.31	0.21
Eigen value	3.53	1.76	0.77	0.54	0.22
% of variation	50.4	25.2	11.0	7.7	3.1

TABLE 5.11 Distribution of phosphorus exported from the flag leaf blade of plants grown with contrasting phosphorus regimes; experiment 3 (% of total exported)

Time after ^{32}P applied to flag leaf blade (hours)	24		168		
Time after anthesis (days)	27		33		
Phosphorus regime	Control	Low P	Control	Low P	s.e.
grain	58.2	44.1	61.6	75.6	5.7
chaff	5.3	7.6	2.4	2.8	1.1
peduncle	5.2	12.8	3.1	4.3	0.9
flag leaf sheath	4.8	8.8	3.2	7.5	1.1
lower stem	10.2	22.4	9.5	6.2	2.1
lower leaves	0.7	0.6	2.1	0.4	0.2
root/crown/tillers	15.8	3.7	18.2	3.1	3.0
Total relative counts	14,218	9,511	29,707	33,062	3,800

TABLE 5.12 Fractionation of total (^{31}P) and labelled (^{32}P) phosphorus in stems 24 and 168 hours after labelling the flag leaf (experiment 2). Values given are the percentage of the total phosphorus in the stem

	P ester (%)		P inorganic (%)		P lipid (%)		P residue (%)		Total ^{31}P (µgP/100 mg DW)	Total ^{32}P (cpm/100 mg DW)	Stem dry weight (mg)
	^{31}P	^{32}P	^{31}P	^{32}P	^{31}P	^{32}P	^{31}P	^{32}P			
Labelling period											
24 hours											
Control	4.4	28.0	37.3	55.1	54.2	10.8	4.1	6.1	861	36,215	0.50
Low P	9.6	21.5	2.5	53.1	66.6	14.3	21.3	11.2	98	36,100	0.75
168 hours											
Control	4.6	7.3	33.8	74.5	57.3	12.8	4.4	5.3	738	84,450	0.46
Low P	11.0	10.0	2.5	36.8	65.8	29.9	20.7	23.3	79	41,100	0.66
Phosphorus	***[1]	n.s.	***	***	*	***	***	***	***	n.s.	**
Labelling period	n.s.	***	n.s.	n.s.	n.s.	***	n.s.	***	n.s.	*	n.s.
Interaction	n.s.	**	n.s.	***	n.s.	***	n.s.	***	n.s.	n.s.	n.s.

[1]Significance of difference between means; n.s., not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

TABLE 5.13 Specific activity of phosphorus fractions of stems

(%^{32}P: %^{31}P in fraction; experiment 3)

Labelling period	P_{ester}	$P_{inorganic}$	P_{lipid}	$P_{residue}$
24 hours				
Control	13.1[1]	1.5	0.20	1.5
Low P	2.3	22.0	0.21	0.5
168 hours				
Control	3.3	2.3	0.26	1.4
Low P	1.0	19.0	0.46	1.1
Phosphorus	n.s.	**	***	***
Labelling period	n.s.	n.s.	***	*
Interaction	n.s.	n.s.	**	**

[1]ratio unreliable due to variation in ^{31}P values.

Significance of difference between means; n.s., not significant;

* P < 0.05; ** P < 0.01; *** P < 0.001.

TABLE 5.14 Fractionation of total (^{31}P) and labelled (^{32}P) phosphorus in grain 24 and 168 hours after labelling the flag leaf (experiment 3). Values given are the percentage of the total phosphorus in the grain

	P inorganic + ester		P phytate		P residue		Total ^{31}P	Total ^{32}P	Grain dry
	^{31}P	^{32}P	^{31}P	^{32}P	^{31}P	^{32}P	(µg P/100 mg DW)	(cpm/2 mg DW)	weight (mg)
Labelling period									
24 hours									
Control	48.4	89.0	33.2	6.3	18.4	4.8	517	687	24.9
Low P	54.2	89.3	16.2	5.4	30.0	5.4	290	321	21.8
168 hours									
Control	40.6	45.6	39.6	40.4	19.8	14.0	455	1168	33.6
Low P	45.7	60.9	24.2	19.0	30.1	20.1	251	1172	28.9
Phosphorus	*	**	***	***	***	**	***	n.s.	
Labelling period	***	***	**	***	n.s.	***	**	***	
Interaction	n.s.	**	n.s.	***	n.s.	*	n.s.	n.s.	

Significance of difference between means; n.s., not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

TABLE 5.15 Specific activity of phosphorus fractions in grain (% ^{32}P: % ^{31}P in fraction; experiment 3)

	P inorganic + ester	P phytate	P residue
Labelling period			
24 hours			
Control	1.84	.187	.26
Low P	1.65	.339	.18
168 hours			
Control	1.12	1.02	.71
Low P	1.34	.78	.67
Phosphorus	n.s.	n.s.	**
Labelling time	***	***	***
Interaction	***	***	n.s.

Significance of difference between means; n.s., not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

CHAPTER 6

LEAF SENESCENCE AND GRAIN DEVELOPMENT: THE EFFECTS OF PHOSPHORUS APPLICATIONS VIA THE FLAG LEAF, GLUMES AND ROOTS DURING GRAIN FILLING

6.1 INTRODUCTION

Increases in grain yield could be achieved by extending the duration of grain filling (Eastin and Sullivan, 1974) or the photosynthetic capacity of the flag leaf (Austin, 1982).

The low P plants in Chapter 4 were dominated by senescence and the photosynthetic capacity of the flag leaf blade rapidly fell to zero before maximum grain dry weight was achieved. This possibly reduced the duration of grain filling and partly accounted for the lower grain yields of the main culm.

For example, a 10% increase in grain yield would result if the linear phase of grain filling was extended by four days in environments which restrict the grain filling period to about 40 days. The length of the grain filling period is regulated by the mean daily temperature; being reduced by 3.1 days for each 1°C rise in temperature (Wiegand and Cuellar, 1981). Cessation of grain dry weight accumulation and the onset of rapid loss of water appear to be associated with the deposition of lipids in the chalazal zone of the grain (Sofield et al., 1977,b). If these processes in the grain are not the primary cause of senescence in low P plants it may be possible to extend the rate and duration of photosynthesis by the flag leaf in plants by either:

(a) seeking plants which export phosphorus slowly, or

(b) timely applications(s) of phosphorus to the foliage.

The former approach has not been examined to date and foliar applications of phosphorus (and other major plant nutrients) have no effect or produce inconsistent yield increases (Ozanne and Petch, 1978; Kannan, 1980; Poole et al., 1983). By applying the nutrients

N, P, K and S during grain filling, Ozanne and Petch (1978) obtained significant increases (10-21%) in wheat yields which they stated could be attributed to an increase in the number of harvestable grains. The phosphorus in the sprays made a smaller contribution to the increased yield than the other nutrients.

From field studies it is not possible to distinguish indirect effects of foliar applications of phosphorus (such as delayed senescence of leaves or glumes) from direct effects of phosphorus on grain development. Jenner (1976) referred to unpublished data of Jenner and Reuter which suggested that grains grew faster when cultured in sucrose which contained 50 mM potassium phosphate compared to just sucrose. However, work by Rijven and Gifford (1983b) indicates the above effect was due to the potassium ions and not to the phosphorus.

Foliar applied phosphorus may enhance grain filling by:

(a) maintaining the exchange of triose-P across the chloroplast envelope, i.e. increase the activity of the "source" as defined by Herold (1980);

(b) promoting the size of the "grain sink", i.e. the number of endosperm cells;

(c) maintaining the activity of the "total sink", i.e. all metabolically active, non-assimilating tissues (Herold, 1980);

(d) reducing the rate of senescence indirectly by promoting cytokinin release from the roots relative to release of abscisic acid production in the shoots (Nooden, 1980; Michael and Beringer, 1980);

(e) a combination of the above factors.

The two studies presented in this chapter were designed to examine suggestions a, b and c.

6.2 MATERIALS AND METHODS

6.2.1 Experiment 1. Mid grain filling applications of phosphorus

Wheat (cv. Kite) plants were grown as described in Chapter 2 at 18°/13°C with Hoaglands nutrient solution containing 1 mM P (control), or 0.25 mM P for 15 days then no P (low P), each morning and water each afternoon. Twenty days after anthesis plants from the two phosphorus treatments were transferred to an artificially lit L.B.H. cabinet (Morse and Evans, 1962) with the same temperature settings as the glasshouse. Plants were given no foliar treatment or phosphorus was applied to the flag leaf or to the glumes of the central spikelets of the ear of the main culm. The effect of one application of 400 µg phosphorus applied via the roots 21 days after anthesis was tested in a set of low P plants which received 0.25 mM P for only 12 days (Figure 6.1).

6.2.2 Experiment 2. Pre and early post anthesis applications of phosphorus

Low P wheat plants were grown as above with 0.25 mM P for 15 days. Commencing at heading and 8 days post anthesis plants were either untreated or phosphorus was supplied to the flag leaf blade or ear (Figure 6.1).

6.2.3 Foliar applications of phosphorus

Using data for low P plants from an earlier experiment the loss of phosphorus from the flag leaf blade was calculated to reach 7 µg P

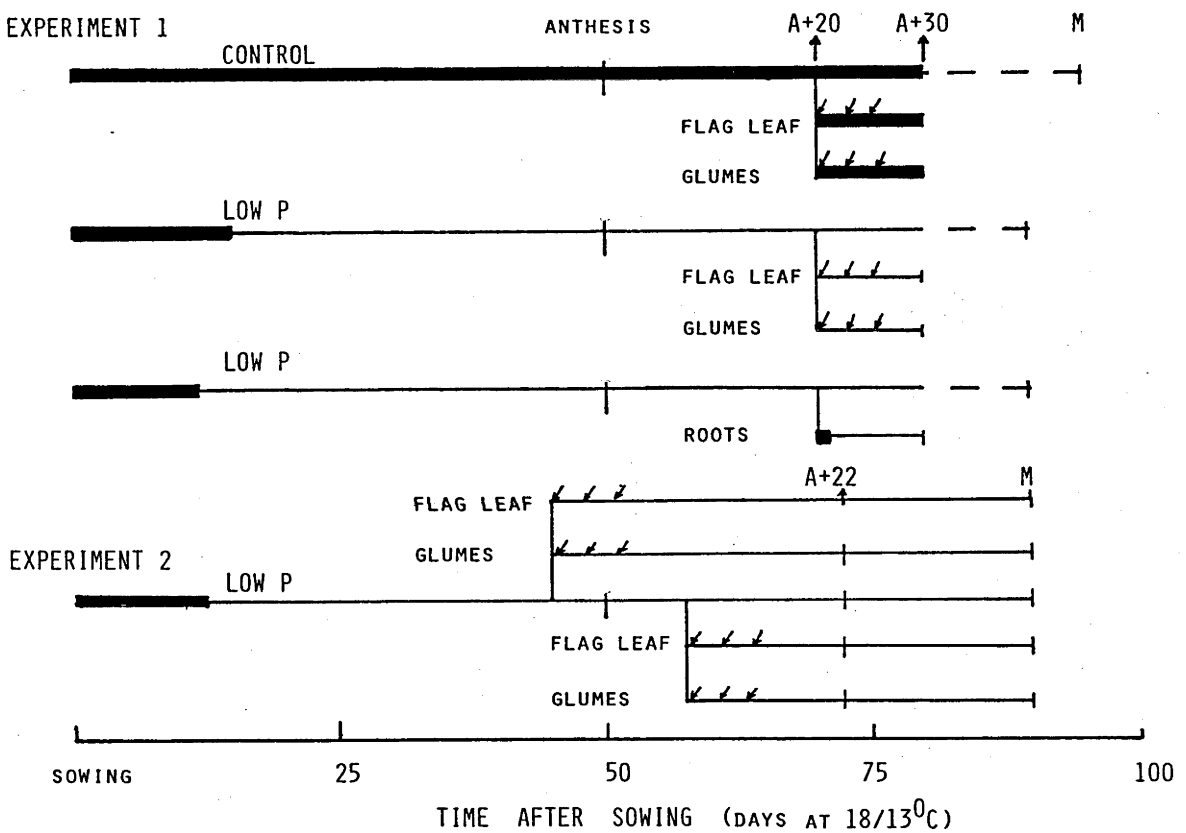


Figure 6.1 Designs of experiments 1 and 2.
Broad lines = daily applications of phosphorus via the roots.
Thin lines = no phosphorus in nutrient solution.
Arrows indicate foliar applications of phosphorus.
A+20, A+22 and A+30 indicate harvest times (days after anthesis) and M = maturity.

day^{-1}. In all foliar treatments here a total of 80 µg P leaf^{-1} was applied in 3 applications over 6 days.

The phosphorus was applied as a 0.08M ammonium dihydrogen orthophosphate solution containing 0.1% Tween 20, and adjusted to pH 6.5 with ammonium hydroxide. This was brushed onto the photosynthetically active portion of the adaxial surface of the flag leaf or the glumes of the central fertile spikelets. A preliminary check confirmed that this phosphorus solution did not damage the wheat leaf (see also Barel and Black, 1979b). The phosphorus applied to the ear was traced using ^{32}P and found to remain predominantly within the ear.

6.2.4 Assessments

Plant senescence was followed by measuring the net carbon exchange (N.C.E.) of the flag leaf (Chapter 2.5.5), or by visually estimating the proportion of the leaf still green and calculating N.C.E. using relationships established in this and an earlier study (Figure 6.2).

Grain dry weight changes were followed by taking grains from the A and B florets of central spikelets before and 10 days after the initial application of phosphorus (Experiment 1), and at 22 days after anthesis and at maturity in Experiment 2. The grains taken 22 days after anthesis were analysed for total phosphorus and nitrogen (Chapter 2.7.1) and the number of endosperm cells was determined by the method of Rijven and Wardlaw (1966).

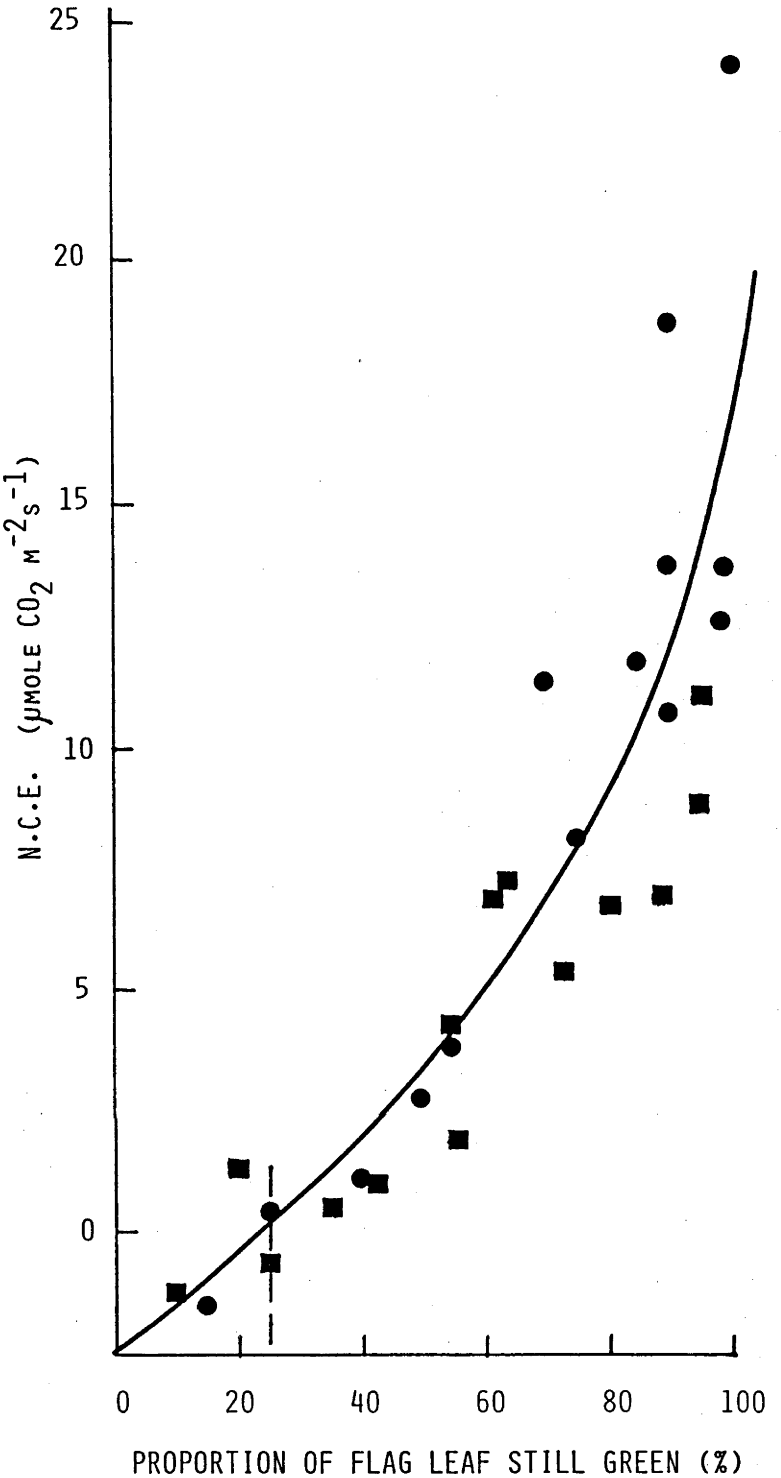


Figure 6.2 Relationship between a visual score of senescence and photosynthesis of the flag leaf of low P plants in experiment 1 of this chapter (■) and experiment 2 of Chapter 3 (●).
Zero N.C.E. occurs when about 25% of the leaf is still green due to the technique used (Figure 4.1).
N.C.E. = $-2.545 + 0.115X - 6.08\times10^{-4}X^2 + 1.31\times 10^{-5}X^3$ where X = % area still green ($R^2 = 0.799$).

6.3 RESULTS AND DISCUSSION

6.3.1 Experiment 1. Mid grain filling applications of phosphorus

The control and low P plants were similar to those described in Chapter 4. In control plants the foliar treatments had no effect on leaf photosynthesis, nitrogen status, grain dry weight or grain phosphorus (Table 6.1). The applications of phosphorus to the flag leaf more than balanced losses which occurred between 20 and 30 days after anthesis. This showed that some phosphorus either did not enter the leaf, or entered the leaf and was not translocated. The actual proportion absorbed was not measured. Other work suggests that it can vary from 13-45% (Alston, 1979) to 97% (Barel and Black, 1979a). Uptake and translocation are greater in a humid atmosphere (Thorne, 1958), with some wetting agents (Koontz and Biddulph, 1958; Bouma, 1969), when a higher concentration of phosphorus is applied (Koontz and Biddulph, 1958; Barel and Black, 1979a), from more acid solutions (Bouma, 1969), from some phosphorus compounds (Barel and Black, 1979a), and by certain plant species (Barel and Black, 1979a).

In low P plants flag leaf photosynthesis declined from 8.4 to less than 1 μmole CO_2 m^{-2} s^{-1} in the untreated plants or where phosphorus was applied to the ear. The flag leaf almost maintained the same rate of photosynthesis when phosphorus was applied to the leaf (Table 6.1). Both the phosphorus and nitrogen concentrations in the treated flag leaf were higher than in the untreated or ear treated plants. Some of the phosphorus was possibly on the surface of the treated leaf, but that which was absorbed reduced the loss of

nitrogen, as seen visually by the loss of chlorophyll, and the rapid decline in photosynthesis in the untreated plants.

Plants given foliar phosphorus via the flag leaf produced grains in central spikelets which were 3.4% heavier (P = .05) at 30 days after anthesis (10 days after beginning the foliar treatments). This was largely due to a lower number of grains per ear in the treated plants. The yield per ear was not increased by the treatments (Table 6.1).

The single application of 400 µg P into the root zone 21 days after anthesis also reduced senescence apparently by maintaining the phosphorus concentrations, and hence nitrogen and chlorophyll in the flag leaf (Table 6.2).

There was no increase in grain dry weight as a result of the delayed senescence but a small increase in the grain phosphorus concentration following this application via the roots.

From the relationship in Figure 6.2 it is evident that the rate of assimilation of carbon by a leaf with only 30% of the surface area still green is low due to the small area of active tissue and the low rate of photosynthesis (2-6 µmole CO_2 $m^{-2}s^{-1}$).

6.3.2 Experiment 2. Pre and early post anthesis applications of phosphorus

Phosphorus applied to the leaves increased the flag leaf area duration[1] by 10% for the period from anthesis to 35 days after anthesis and by 21% between 15 and 35 days after anthesis. This was due to delayed senescence between 15 and 30 days after anthesis. All flag leaf blades were dead 35 days after anthesis. Applications to

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[1]Flag leaf area duration = green flag leaf area integrated over time.

the glumes kept the glumes greener for several days longer but did not affect the flag leaf (Figure 6.3).

Data for the grain of the main culm are presented in Table 6.3. At 22 days after anthesis (mid grain filling and 8 days after the last post anthesis phosphorus application) the central spikelet grains had similar dry weight in each treatment. The concentration and absolute amount of phosphorus per grain were higher in the treated plants than in the untreated and higher where the phosphorus was applied to the glumes compared to the flag leaf blade.

Grain number per ear, grain nitrogen, and the number of endosperm cells were not affected by the treatments.

At maturity the grain yield per ear (main culm) was not increased over the untreated but the phosphorus applied to the glumes prior to anthesis gave a 6.3% increase in the weight per grain for central spikelets and a 5.4% increase in the average weight of all grains in the ear ($P = 0.05$). The latter measurements had smaller coefficients of variation; 11.8% for yield, and 11.6 for grain number, but only 7.0% for mature weight grain^{-1} of central spikelets and 6.9% for the average weight of all grains in the ear of maturity.

6.3.3 General discussion

The foliar applications of phosphorus in these two studies with wheat led at best to only small (< 6.3%) increases in the dry weight of grains. While the yield increases were not encouraging, the foliar applications allow comments on the role of phosphorus in senescence and grain filling.

It appears that the phosphorus applied to low P plants in the present experiments offset the breakdown of photosynthetic tissues

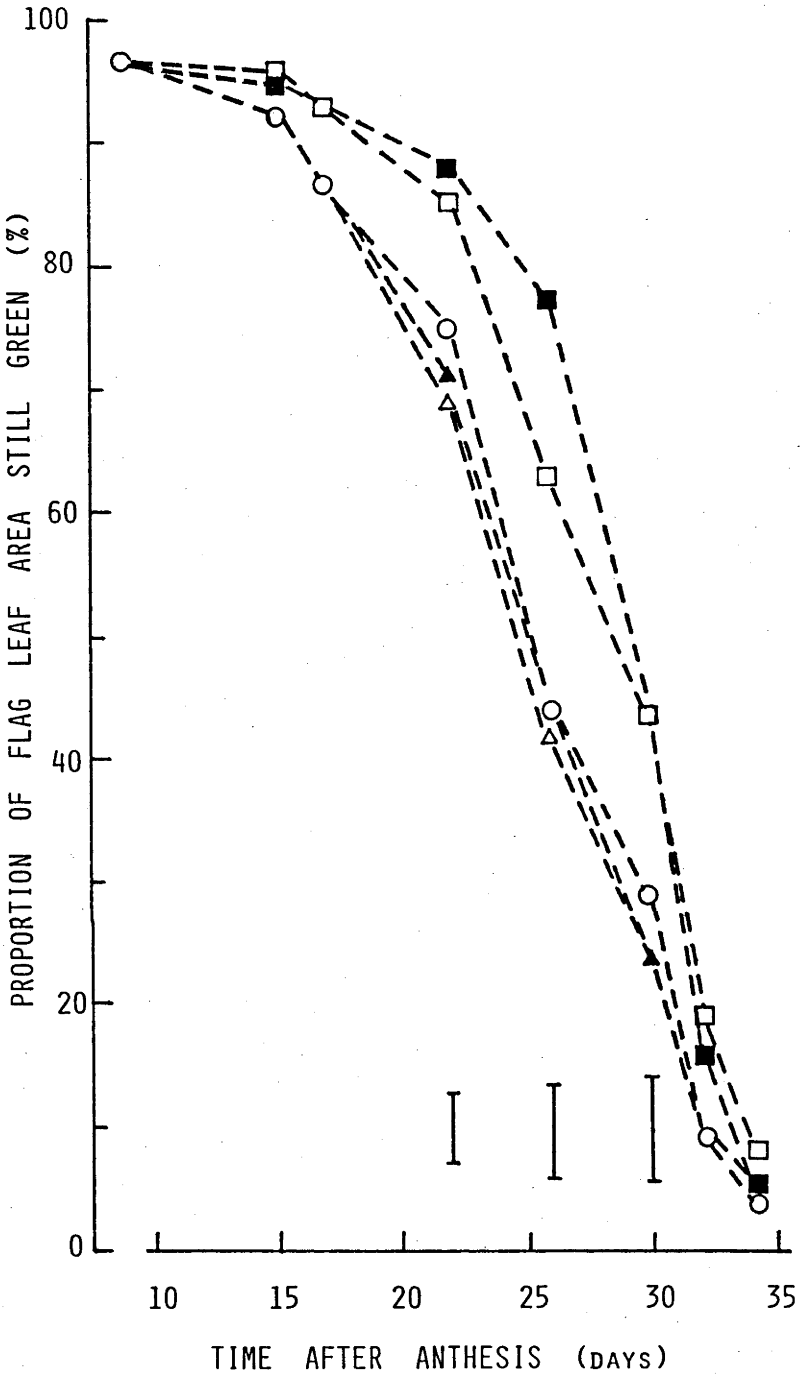


Figure 6.3 Flag leaf area duration in experiment 2 by visual assessment. Means and 2X standard error bars are for data adjusted for actual anthesis date using covariance analysis. □■, P to flag leaf; △, ▲ P to glumes prior to and early post anthesis respectively and ○ untreated.

caused by export of phosphorus. However, after anthesis photosynthesis (N.C.E.) by senescing leaves of low P plants is low relative to control plants (Chapter 4). The 10 to 20% increase in flag leaf area duration obtained here was apparently insufficient to maintain both the metabolism of the flag leaf and to provide additional assimilate for the grain.

In soybeans Sesay and Shibles (1980) increased leaf nutrient levels but found the photosynthetic rate, soluble protein and chlorophyll levels declined at the same rate as in control plants (no foliar nutrients applied). Abu-Shakra et al. (1978) and Nooden et al. (1979) reported that delaying senescence does not necessarily lead to increased yields.

In Chapter 3 (Experiment 1) phosphorus supplied after anthesis did not promote grain yield. Similarly in Experiment 2 plants with higher flag leaf phosphorus concentrations at anthesis exported phosphorus more rapidly, but did not have higher yields. These experiments support the thinking that neither the nutrient concentration in the leaf at anthesis nor senescence due to nutrient stress, are primary factors in determining yield and that the components of yield are "fixed" at an early stage.

This study also failed to demonstrate any direct effects of phosphorus on grain size. In view of the low level of inorganic + ester phosphorus in the grain reported in Chapter 4, especially during the phase of endosperm cell proliferation, and the 12-14% increase in grain phosphorus at day 22 here, it is concluded that grain phosphorus is not a primary factor causing the lower rate of grain dry weight accumulation shown in Chapter 4. This supports Rijven and Gifford's (1983b) finding that phosphorus does not limit

starch production. Raising the phosphorus level in the grain does not appear to modify the hormone balance and thereby delay senescence and increase grain yield. Only a small proportion of the phosphorus leaving the flag leaf is directed to the roots (Chapter 5). If a foliar application of phosphorus is to stimulate cytokinin production in, and/or export from roots (Menary and van Staden, 1976), then larger applications of phosphorus may be needed.

In this study with foliar applications and previous studies where phosphorus was taken up from the soil during grain development (Michael et al., 1980; Piper and de Vries, 1964) the concentration of phosphorus in grain increased without, or at a greater rate than, any increase in grain yields. This is inefficient use of phosphorus (grain yield:phosphorus in grain).

The ideal situation in which foliar applications of phosphorus could be recommended, would be one in which significant increases in yield are obtained consistently without an increase in grain phosphorus concentration.

Alternative strategies, such as applying additional phosphorus at planting and seeking genetically efficient plants (those which produce more grain per unit of phosphorus acquired from the soil) could be of greater benefit in the short and longer terms respectively.

Nooden and Leopold (1978) and Biswas and Choudhuri (1980) reported that the balance between phytohormones (cytokinins and abscisic acid) may ultimately regulate senescence in nutrient stressed plants. A better understanding of the interactions between phytohormones and senescence could explain the present findings and lead to more efficient utilization of phosphorus.

Table 6.1 Responses by wheat to foliar applications of phosphorus 20 days after anthesis in experiment 1

Days after anthesis:	20	30		
	Initial sampling	Untreated	P to flag	P to ear
Flag leaf				
N.C.E.[1]				
Control	19.5 (0.7)[2]	17.3 (1.1)	18.0 (1.1)	18.6 (1.1)
Low P	8.4 (1.0)	0.7 (0.6)	6.0 (0.7)	0.1 (0.7)
n[3] =	5		4	
Total P (%)				
Control	0.62 (0.04)	0.55 (0.04)	0.66 (0.04)	0.50 (0.03)
Low P	0.09 (0.00)	0.05 (0.01)	0.13 (0.00)	0.05 (0.01)
n =	6		5	
Total N (%)				
Control	4.09 (0.21)	3.68 (0.15)	3.50 (0.16)	3.66 (0.15)
Low P	3.10 (0.21)	1.43 (0.29)	2.64 (0.12)	1.56 (0.29)
n =	6		5	
Central spikelet grains				
Dry weight				
Control	20.1 (1.2)	35.2 (1.0)	34.0 (1.0)	35.1 (0.7)
Low P	17.5 (1.1)	26.7 (0.05)	27.6 (0.4)	26.9 (0.5)
n =	6		11	
Total P (%)				
Control	0.46 (0.01)	0.44 (0.00)	0.45 (0.01)	0.44 (0.00)
Low P	0.20 (0.01)	0.18 (0.00)	0.18 (0.00)	0.19 (0.00)
n =	6		5	
Whole main culm ear				
Number of grains		36 (1.5)	32 (2.0)	34 (1.7)
Average grain weight (mg)		22.0(0.5)	23.4 (1.0)	22.2(0.5)
Yield (g)		0.80(0.04)	0.75(0.06)	0.75(0.04)
n =			11	

[1] µmole $CO_2 m^{-2} s^{-1}$

[2] standard error of mean

[3] n = number of samples

Table 6.2 Responses by low P plants to phosphorus applied to the roots 21 days after anthesis, experiment 1

Days after anthesis	21	28	31
Flag leaf			
Green area (%)			
Untreated	80 (8)[1]	11 (3)	2 (0.3)
+ 400 µg P on day 21	84 (6)[2]	37 (6)	13 (5)
Phosphorus (% DW)			
Untreated	0.08 (.0)		0.02 (.0)
+ 400 µg P on day 21			0.05 (0.2)
Nitrogen (% DW)			
Untreated	3.06 (0.16)		0.81 (0.08)
+ 400 µg P on day 21			1.96 (0.54)
Central spikelet grains			
Dry weight (mg grain^{-1})			
Untreated	20.9 (1.5)		28.6 (0.5)
+ 400 µg P on day 21			29.3 (1.5)
Phosphorus (% DW)			
Untreated	0.160 (0.00)		0.165 (0.01)
+ 400 µg P on day 21			0.170 (0.01)

(8)[1,2] standard error based on 6 and 7 plants respectively.

TABLE 6.3 Responses in grain to foliar applications of phosphorus in Experiment 2.

	Samples taken from central spikelets 22 days after anthesis					Maturity			
								Dry Weight (mg grain^{-1})	
	Dry Weight (mg grain^{-1})	P (%)	P (ug grain^{-1})	N %	Endosperm cells (x1000 grain^{-1})	Number (grains ear^{-1})	Grain Yield (g ear^{-1})	2 Central Spikelets	Average all grains in ear
Untreated	19.0	0.233	43.8	2.27	48	38	1.16	38.4	30.6
Preanthesis applications									
to flag	19.5	0.245	47.5	2.24	42	37	1.16	39.1	31.8
to glumes	19.6	0.254	49.7	2.26	48	37	1.19	40.8	32.2
Early Post anthesis applications									
to flag	19.3	0.244	46.9	2.26	45	36	1.13	38.4	31.2
to glumes	19.4	0.257	49.4	2.34	50	38	1.15	38.5	30.1
s.e.	0.5 ns	0.006**	1.7**	0.04 ns	5 ns	1.4 ns	0.05 ns	0.9*	0.7*

Significance of difference between means; n.s., not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

CHAPTER 7

GRAIN PHOSPHORUS IN WHEATS OF THREE PLOIDY LEVELS WHEN GROWN WITH CONTRASTING PHOSPHORUS REGIMES

7.1 INTRODUCTION

The concentration of phosphorus in the grain of modern wheats varies from 0.12 to 0.56% of the dry weight (Williams and Colwell, 1977) and up to 0.76% when grown at high temperatures in the glasshouse (Sofield et al., 1977b). This range can be accounted for by variation in the supply of phosphorus from the soil and fertilizer (Colwell, 1963; Osborne et al., 1977; previous chapters of this thesis etc.), seasonal conditions (Ferguson, 1964; Piper and de Vries, 1964; Sofield et al., 1977b; Osborne et al., 1977), and sowing date (Batten and Khan, unpublished data). Genetic variation accounts for a smaller proportion of the range in grain phosphorus. It appears from reports by Lipsett (1964, 1969) and Batten and Khan (1982 and unpublished data) that grain phosphorus concentration is inversely related to yield and harvest index in a similar pattern to that for grain protein (Kramer, 1979). If true genetic differences in grain nitrogen content can only be compared at similar yields or harvest indexes, then caution may be required when interpreting differences in grain phosphorus concentrations.

Recently there has been some interest in using genes of the ancestors of modern wheats to improve grain protein in bread wheat (Law, 1982; Dr A. Blum, Israel, personal communication). This approach may also be useful for improving the efficiency of utilization of phosphorus. Some of the tetraploid wheats for example have very large grains (Halloran and Pennell, 1982) and these may have lower concentrations of phosphorus (i.e. a higher carbon:phosphorus deposition rate). Thus, an examination of a diverse range of genetic material appeared warranted and this has been undertaken in the work reported below. Wheats from each of the three ploidy levels

and from different breeding programs have been compared at contrasting levels of phosphorus supply. Efficient utilization of phosphorus is considered both in terms of an ability to produce a high yield under a low supply of phosphorus and to not accumulate phosphorus to excess under a high supply of phosphorus.

Phosphorus is found as phytate in the aleurone and embryo regions of grain in globoid crystals within the protein bodies. Energy-dispersive x-ray analysis (EDX) of these crystals reveals predominantly phosphorus, potassium and magnesium (Lott and Spitzer, 1980). As there are no reports of the effect of phosphorus supply on the composition of phytate the opportunity was taken to examine the ultrastructure and mineral composition of aleurone cells from control and low P wheat.

7.2 MATERIAL AND METHODS

7.2.1 Experimental conditions

Twenty lines of wheat (Table 7.1) were germinated at room temperature then both winter and spring types were vernalized for 9 weeks at 2°C. The seedlings were grown as described in Chapter 2 in a glasshouse controlled to 15°C during the day and 10°C at night. Two phosphorus regimes were supplied, control (1 mM P daily) and low P (0.25 mM P for 21 days then nil P).

Control plants were detillered regularly to the main culm plus one tiller to reduce shading and facilitate watering. Low P plants were detillered only at anthesis. Within each P regime there were six blocks of the 20 lines.

7.2.2 Lines of wheat grown

The wheats selected included diploid, tetraploid and hexaploid

types (Table 7.1). These provided a wide genetic base especially with respect to seed size and harvest index. Some of the lines were those studied by Evans and Dunstone (1970). Two turgidum wheats, Calibasan and Levissimum, and three vulgare wheats, Spica, Keowechen and Gigue were chosen because they produce large seed (K. Syme, personal communication).

Kite and Gabo were included as standard local genotypes. The oligoculm was included because it produces only one culm under certain conditions (see Appendix 9). With this feature it conforms to Donald's (1968) model and is potentially a valuable type for nutrition studies where detillering could confound the experiment.

Two Brazilian wheats, Cotipora and Carazinho, were included because they were selected on acid-low P soils.

7.2.3 Examination of the grain

When the ears matured the plants were dried and the shoot and grain of the main culm weighed for yield and harvest index. The grain from five replicates was analysed for total phosphorus and nitrogen (Chapter 2.7).

Several grains for the cultivar Kite were examined using transmission electron microscopy (TEM) and EDX analysis as described below.

7.2.4 Ultrastructure of aleurone cells in dry wheat

Traditional aqueous fixation procedures brought about the loss of globoid crystal material, especially from the aleurone cells of low P wheat. As a result an alternate procedure was used to retain as much phytate as possible.

Control and low P wheat grains were de-embryonated and then portions of the grain exterior, including the aleurone layer, were dissected from the bulk of the endosperm. Pieces from individual grains were kept separate. Dry tissue was fixed for 4 hr in cold 5% glutaraldehyde in 60% ethanol at neutral pH. Following fixation the tissue was gradually dehydrated to absolute ethanol, further dehydrated with propylene oxide, infiltrated with Spurr's epoxy resin and hardened. Blocks of tissue were cut dry with a Reichert 0m UZ ultramicrotome. Sections, 0.5 to 1.0 µm thick were picked up from the knife edge with an eyelash and placed onto parlodion-carbon coated grids moistened with 95% ethanol. Sections were viewed, without any post-staining, in a JEOL JEM-100S transmission electron microscope operating at 80 or 100 KV.

7.2.5 Energy dispersive x-ray analysis

Aleurone tissue samples of low P and control wheat grains were dissected as described previously. Tissue pieces were then chopped to a powder using a razor blade. A Formvar-carbon coated copper grid was smeared with the powder and any unattached particles were shaken loose. The grid in a carbon holder was inserted into a JEOL JEM-100CX transmission electron microscope operating at 80KV. Round shape and density were used to locate globoid crystals which are naturally electron dense. Elemental composition of globoid crystals was determined using a KEVEX 5100 energy dispersive x-ray analysis system.

Each globoid crystal was analysed for 100 seconds. Major elements present, principal emission lines of emission levels in KeV are as follows: magnesium K_a at 1.253; phosphorus $K_{a1,2}$ at 2.013

and K_{a4} at 2.028; potassium $K_{a1,2}$ at 3.312 and K_b at 3.589 (10% of $K_{a1,2}$ peak). Copper peaks (K_b at 8.904) are artifacts of copper support grid usage.

7.3 RESULTS

7.3.1 General plant growth

Data for each line grown with the control and the low P regime are presented in Table 7.2. As ploidy increased from diploid to hexaploid fewer tillers were produced, the time to anthesis decreased and the duration of grain filling increased.

Control plants, except for the oligoculm (28)continued to produce tillers after being detillered at anthesis. Except for the diploid wheat the low P plants produced few tillers after anthesis.

With the control phosphorus regime the hexaploid wheats generally set more grains but the durum Kubanka W8 (16) had the highest grain number (73 ear^{-1}). With the low P regime the oligoculm (28) set 54 grains ear^{-1} which was 81% of the set by control plants. Polish wheat (21) and the Brazil ian line Carazinho (30) set < 20% of the grains set by control plants, while T. monococcum W292 (11) was almost sterile. Average weight $grain^{-1}$ was reduced to 50 to 90% of that of control grains in low P plants.

At maturity, grain yield of the main culm ranged from 0.003 g for line 11 to 2.60 g for the oligoculm (28) in the low P set and from 0.15 g for line 7 to 6.155 g for the oligoculm grown with the control phosphorus regime.

Harvest index increased with ploidy level while grain phosphorus and nitrogen concentrations declined. The absolute amounts of

phosphorus and nitrogen were highest in the larger grains of the durum (22), Polish (21) and oligoculm (28) wheats.

Table 7.3 contains correlation matrixes for the control and the low P series (r values for simple linear regressions). Grain yield (for the main culm) increased with the biological yield, the harvest index, the average grain weight and the number of grains ear^{-1}. Plots of mean values for each line showed that harvest index reached a maximum value of about 59% where biological yield was about 5 g $culm^{-1}$ and where the ear set 50 grains (Appendix 10). Plants which produced more tillers, were taller, or took longer to reach anthesis had lower yields. Examples of those are evident in Table 7.2.

Grain phosphorus was positively correlated with plant features associated with low yields and negatively correlated with grain yield, biological yield, harvest index, average grain weight and grains ear^{-1}.

In low P plants the proportion of the flag leaf area which was green at anthesis was also positively correlated with yield and yield components, and negatively correlated with grain phosphorus concentration.

7.3.2 Grain total phosphorus and nitrogen

Plots of grain phosphorus against grains ear^{-1}, average grain weight and grain yield (Appendix 10.6-10-8) showed that these were curvilinear relationships. At low P the oligoculm (28) had a higher grain number and grain yield than other lines with the same phosphorus concentration. One durum and the Polish wheat (22,21) and two bread wheats (26 and 30) had relatively few grains with higher phosphorus concentrations than the general trend. In the control set lines 21, 22 and 28 had larger grains than other lines with similar grain phosphorus. Ukraine (26) which set only 19 grains

ear^{-1} with an average weight of 59 mg grain^{-1} had a lower grain phosphorus than other lines which set few grains (Appendix 10.6).

The strongest linear correlations with grain phosphorus in Table 7.3 were with harvest index. These are plotted in Figure 7.1. A minimum grain phosphorus concentration of 0.10% is suggested for the low P grains.

The absolute amounts of phosphorus per grain are plotted against average grain weight in Figure 7.2. In the control set phosphorus increased linearly with grain weight. In the low P set there was no mean increase in phosphorus as the average grain weight increased from 20 to 60 mg (except for lines 26 and 30 which set few grains). Gabo (19) and Kite (20), which had high harvest indexes, had less phosphorus grain^{-1} in this group.

Grain nitrogen (μgN grain^{-1}) increased linearly in relation to grain weight regardless of the phosphorus treatment (Table 7.2).

7.3.3 The ultrastructure and EDX analyses of aleurone cells

Aleurone cells from grains produced under the control P regime contained protein bodies with mostly large electron dense globoid crystals. In contrast the protein bodies of low P grains contained numerous small, less dense globoid crystals. These appeared to be concentrated around the perimeter of the protein body (Plate 1, a and b).

EDX analyses revealed that the globoid crystals of both the control and the low P grains contained predominantly phosphorus, potassium and magnesium, with only traces of sulphur, calcium, iron.

Relative to the phosphorus peak the height of the magnesium peak varied in both control and low P samples. The potassium peak

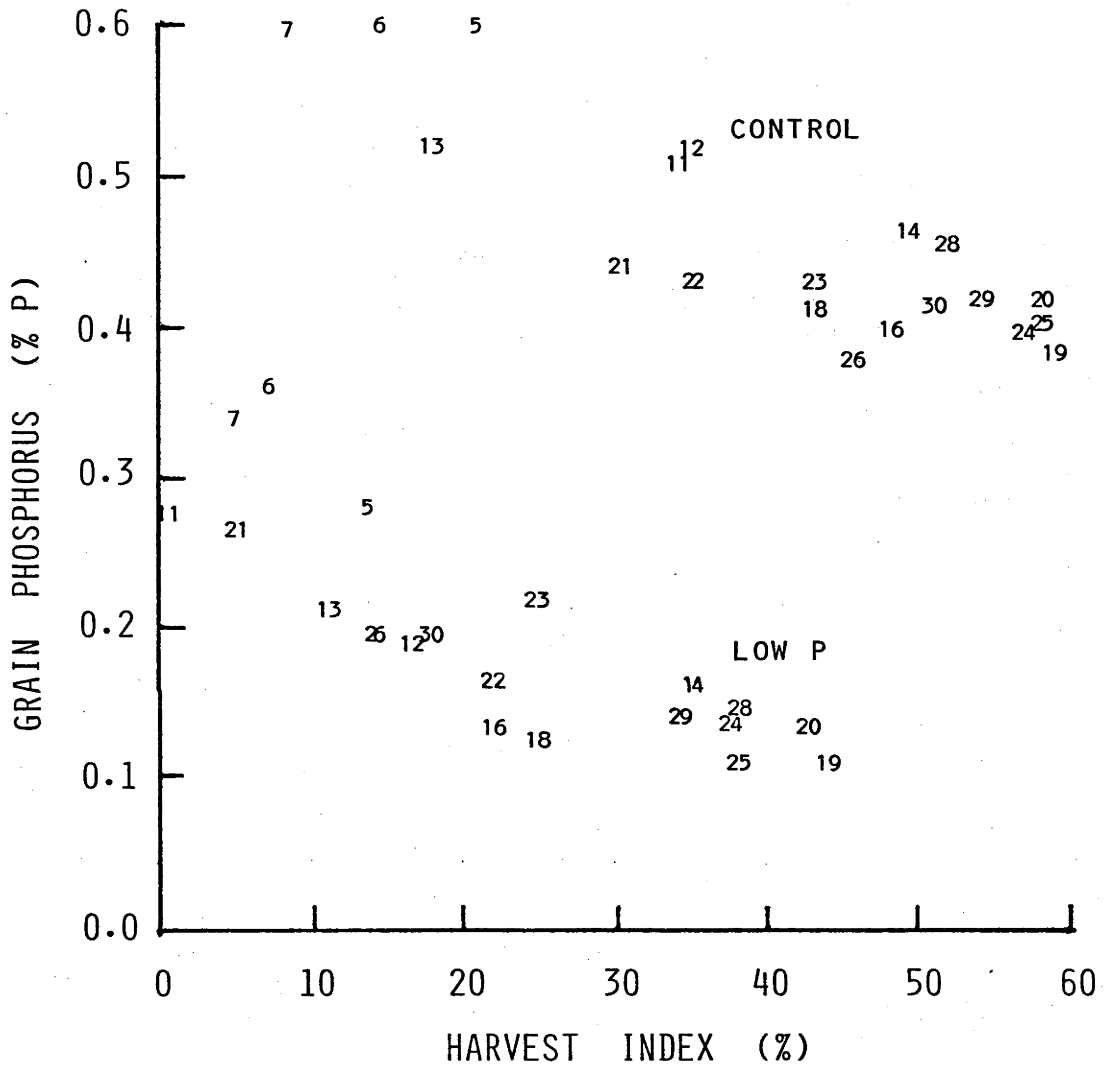


FIGURE 7.1 Relationships between grain phosphorus concentration and harvest index in the main culm of wheat grown with two phosphorus regimes. Numbers refer to the genotypes described in Table 7.1.

Harvest Index = grain : grain + straw dry weight ratio

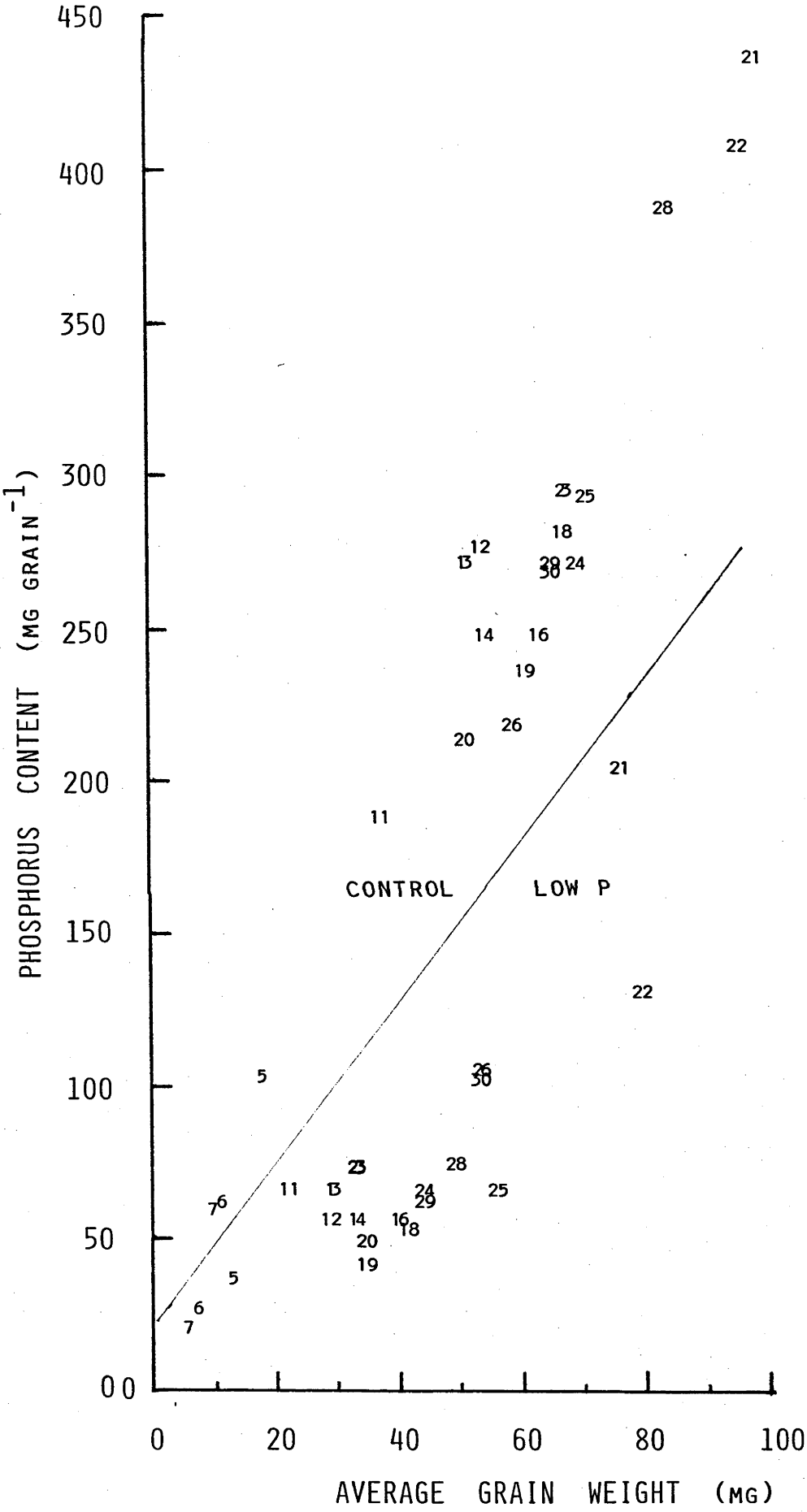


FIGURE 7.2. Relationships between grain phosphorus content and grain size in wheat grown with two phosphorus regimes. Numbers refer to the genotypes described in Table 7.1.

was always lower than the phosphorus peak in the control but in most low P samples the potassium peak was higher than the phosphorus peak (Plate 1, c and d).

7.4 DISCUSSION

With few exceptions, hexaploid wheats produced more grain yield, had a higher harvest index and lower grain phosphorus concentration than diploid and tetraploid wheat at each level of phosphorus supply.

There is no evidence in the present data to suggest that progenitors of modern wheat are more phosphorus efficient than today's wheats.

This study confirms and expands on the earlier work by Evans and Dunstone (1970), and Bykov et al. (1980) who found that wheat has evolved to produce higher yields through an increase in grain size, grain ear^{-1}, plant weight, the area of the flag leaf and harvest index. Davidson and Birch (1980) and Austin (1980) have shown that breeding has led to higher yields via higher harvest indexes in modern bread wheats. However within each ploidy level considerable variation exists in grain set, grain weight and harvest index. Part of this variation may be due to specific growth conditions, such as temperature, sward density, pot size etc., which disfavour certain genotypes. Here vernalization or the relatively low glasshouse temperature (15°/10°C) may have adversely affected grain set in some lines. On the other hand, the highest harvest index, 58.6% for Gabo, is close the maximum harvest index of 62% suggested by Austin (1982). The oligoculm wheat, which was uniculm under the conditions of this experiment, produced the highest yields but not by way of

the highest harvest index as expected from the work of Atsmon and Jacobs (1977). Here T. monococcum ssp. monococcum (11) yielded very poorly when grown with the low phosphorus regime. This result suggests that Percival (1921) was not referring to low phosphorus soils when he stated that "..... T. monococcum is resistant to frost, drought, rust, poor soils and degradation of birds."

The Brazilian line Carazinho (30) also grew poorly at low phosphorus producing a harvest index of only 17.7%.

It does appear however that the major improvements in efficiency have been achieved already. The trends in the above data suggest that grain phosphorus concentrations will not be reduced further if yield, harvest index, grains ear^{-1} and average grain weight are increased by breeding for these traits. The relationships suggest that phosphorus per se may be approaching the critical value for grain growth. In Chapter 4 grains with 0.15% P at maturity were free of phytate phosphorus during most of the grain development period. This suggests that all the phosphorus in the grain was required for metabolic or structural purposes. In the present experiment Gabo (19) and Gigue (25) had only 0.11% P at maturity.

Kite (20) with 0.14% total phosphorus had some phytate in the aleurone cells at maturity (cf. Figures 4.11, 4.12) but even with the daily applications of all other essential nutrients the composition of the globoid crystals was virtually the same as in control grains. This suggests that the phytate forms when more phosphorus is available than is required for metabolism in the endosperm, rather than the converse that phytate forms the chelate cations, such as magnesium when chlorophyll breakdown occurs (Cosgrove, 1980a).

Further studies are required to define the actual differences in efficiency between lines such as Gabo, which had the highest harvest index, and Gigue, which gave the same grain %P at a lower harvest index. Gabo may maintain a greater rate or duration of photosynthesis to achieve a higher harvest index or Gigue may retranslocate less phosphorus per unit of carbon to achieve a lower grain phosphorus.

Harvest index of plants grown at low P was considerably less than in the control plants, e.g. 43.9 vs 58.6 for Gabo at low and control P respectively. Herein lies an important challenge, namely to raise the harvest index of plants grown with low levels of phosphorus, and thereby increase the grain yield per unit of phosphorus in the plant. A major breakthrough in this respect has been the incorporation of semi dwarf genes into modern wheats. The harvest index of semi dwarf wheat is higher than that of taller wheats at low and higher levels of applied phosphorus (Jessop et al., 1983; pot experiment, and Batten et al., 1984; field experiments).

It may be more rewarding to select for low grain phosphorus at a given harvest index. This is a direct approach which requires a greater work load than the former. However, from Figure 7.1 at low P, lines 25, 24 and 28 had a similar harvest index of 38% but a 30% range in grain phosphorus of 0.111 to 0.144% (P = 0.01). The oligoculm (28) gave very high yields ear^{-1}. There are indications that crosses from uniculms or oligoculm may raise harvest index (Merritt, 1982) and increase yields (Atsmon, personal communication) and so it may be possible to combine genotypes for high harvest index, low grain phosphorus and the oligoculm-uniculm habit to produce a high yielding and phosphorus efficient wheat.

Ukraine (line 26) also appeared to be an efficient wheat at high levels of phosphorus because it had a low grain phosphorus concentration relative to harvest index, or yield.

While this study has revealed several genotypes worthy of further examinations the mechanism(s) which link the translocation of phosphorus and carbon from vegetative tissues to the grain remain unexplained. The nett result of a higher harvest index is a higher transfer of carbon than of phosphorus to the grain. This may be partly because of a smaller straw phosphorus reserve and partly due to an increased rate and duration of assimilation by plants with a higher harvest index. The former is not reported here because the straw samples have not been analysed but at low P the green area of the flag leaf was positively correlated with yield components and harvest index and negatively correlated with grain phosphorus and nitrogen concentrations. Lines which have higher yields may retain phosphorus in vegetative tissues, such as the flag leaf, and thereby prolong photosynthesis and minimize grain phosphorus.

Plant phosphorus distribution, and grain total phytate and cations will be analysed later and reported elsewhere.

TABLE 7.1 Classification of *Triticum* and *Aegilops* lines

	Ploidy/classification[1]	Common name	Genome	Line	Habit[2]	Source of seed
DIPLOID (2n = 14)	*Triticum monococcum*	einkorn wheat				
5	ssp boeoticum (Boiss.) L. et L.	wild einkorn wheat	A	TB1	W	a[3]
11	ssp monococcum L.	cultivated einkorn wheat	A	W292	S	a
6	*Aegilops speltoides* Tausch	goat grass	B	AS1	W	a
7			B	6001	W	a
TETRAPLOID (2n = 28)	*Triticum turgidum* (L.) Thell.	emmer wheat				
12	ssp dicoccoides (Korn.) Thell.	wild emmer wheat	AB	W1043	W	a
13			AB	T6252	S	a
14	ssp dicoccum (Schrank) Thell.	(True) emmer wheat	AB	Khapli W12	S	a
	ssp turgidum					
16	conv. durum (DESF.) MK	macaroni wheat	AB	Kubanka W8	S	a
22			AB	Calibasan	S	AUS20678[4]
21	conv. polonicum (L.) MK	Polish wheat	AB	Levissimum	S	AUS3823
HEXAPLOID (2n = 42)	*Triticum aestivum* (L.) Thell.	dinkel wheat				
18	ssp. spelta (L.) Thell.	spelt wheat	ABD	H2	S	a
19	ssp. vulgare (Vill.) MK.	bread wheat	ABD	Gabo	S	Dr Wardlaw
20				Kite	S	Previous expt.
23				Keowechen	S	AUS402
24				Gigue	S	AUS18135
25				Ukraine	S	AUS3477
26				Oligoculm 112-76	S	AUS20431
28				Cotipora	S	B.Scott, Wagga
29				Carazin ho	S	" " "
30						

1 Following Mac Key (1977)

2 S, spring; W, winter

3 On hand in Phytotron from studies by Evans and Dunstone (1970)

4 AUSTRALIAN WHEAT COLLECTION number

TABLE 7.2 Data for 20 lines of Triticum and Aegilops grown at control and low P regimes

	Anthesis (days after sowing)		Tillers[1] (per plant at anthesis)		Duration of grain filling (days)		Height (cm)		Average grain weight (mg)		Grains ear^{-1}	
	Control	Low P	Control	Low P	Control	Low P	Control	Low P	Control	Low P	Control	Low P
Diploid												
5	92	93	18	10	60	52	115	86	18	13	25	14
11	86	110	34	12	55	55	121	84	37	22	35	<1
6	95	102	25	21	72	69	106	77	11	7	26	7
7	93	92	27	18	64	63	105	83	10	6	14	5
Tetraploid												
12	86	80	21	8	59	55	113	86	54	29	37	14
13	72	73	17	8	63	55	103	79	52	30	17	7
14	63	62	18	5	72	62	68	59	54	33	26	17
16	78	80	10	5	66	59	116	109	63	40	73	23
22	73	77	6	4	76	56	105	98	96	79	33	14
21	95	99	7	3	64	43	129	116	99	76	23	4
Hexaploid												
18	73	78	12	6	77	64	102	85	67	41	60	25
19	57	57	10	5	77	64	67	58	61	35	47	31
20	63	63	9	3	73	58	52	46	51	35	38	23
23	66	67	7	3	75	63	80	70	67	33	30	17
24	66	69	10	5	71	58	88	74	69	44	46	23
25	66	67	7	4	68	62	65	64	70	56	54	19
26	67	63	10	6	66	59	75	61	59	53	19	5
28	68	68	1	1	66	55	64	61	84	49	67	54
29	66	72	9	4	65	59	87	78	65	44	51	20
30	64	64	9	4	70	63	95	87	65	53	58	11
s.e.	3	3	3	1	4	4	6	5	3	3	6	3

1 Tillers per plant prior to detillering at anthesis

TABLE 7.2 (Contd.)

	Biological yield (g main culm^{-1})		Grain yield (g ear^{-1})		Harvest index (grain biological yield^{-1}; %)		Grain phosphorus (% P)		Grain nitrogen (% N)		Grain P (ug grain^{-1})		Grain N (ug grain^{-1})	
	Control	Low P	Control	Low P	Control	Low P	Control	Low P	Control	Low P	Control	Low P	Control	Low P
5	2.347	1.339	0.443	0.176	21.3	13.7	0.600	0.280	4.07	4.05	103	37	696	557
11	3.747	1.033	1.282	0.003	34.2	0.4	0.513	0.318	3.38	2.68	188	65	1241	526
6	1.985	0.675	0.283	0.028	14.6	7.2	0.597	0.360	3.95	5.05	61	26	424	288
7	1.397	0.724	0.152	0.036	8.6	4.8	0.599	0.344	4.08	3.72	60	21	410	231
12	5.823	2,435	1.995	0.338	35.2	16.4	0.519	0.190	3.89	3.81	276	55	2076	1086
13	4.725	1.991	0.863	0.217	18.1	11.0	0.521	0.214	4.46	4.00	273	65	2324	1202
14	2.968	1.608	1.391	0.568	49.4	35.4	0.464	0.163	3.21	3.51	247	56	1718	1172
16	9.517	4.311	4.549	0.913	47.8	21.9	0.402	0.134	3.17	2.89	247	55	1945	1135
22	9.270	4.832	3.175	1.059	35.2	21.6	0.435	0.165	3.76	2.85	407	131	3512	2182
21	8.048	4.494	2.403	0.266	30.2	5.4	0.441	0.269	3.66	3.30	437	204	3621	2438
18	9.451	3.998	4.054	1.003	42.9	25.1	0.415	0.129	3.20	2.84	280	53	2154	1143
19	4.906	2.572	2.873	1.125	58.6	43.9	0.387	0.114	2.53	2.57	237	42	1556	900
20	3.469	1.858	2.026	0.790	57.8	42.4	0.420	0.136	2.97	2.93	213	49	1501	1014
23	4.264	2.096	1.967	0.506	46.5	24.4	0.432	0.219	2.97	2.97	287	73	1982	976
24	5.694	2.688	3.219	1.002	56.7	37.3	0.401	0.140	2.65	2.84	273	65	1805	1256
25	6.449	2.783	3.736	1.049	58.1	37.8	0.407	0.111	2.67	2.59	291	64	1910	1443
26	2.409	1.393	1.165	0.234	45.6	14.2	0.380	0.198	2.91	2.93	218	105	1663	1517
28	11.912	6.906	6.155	2.599	51.8	37.7	0.458	0.144	3.65	3.29	385	73	3059	1603
29	6.152	2.459	3.297	0.845	53.7	34.0	0.423	0.142	2.85	2.94	272	63	1832	1251
30	7.375	3.091	3.767	0.571	50.9	17.7	0.418	0.197	2.84	3.27	270	104	1832	1676
s.e.	0.595	0.243	0.333	0.106	2.9	2.9	0.016	0.018	0.16	0.14	140	10	114	112

TABLE 7.3 Correlation matrixes for parameters in control plants (A) and low P plants (B).
df = 76; > 0.23 = $P_{0.05}$, r > 0.30 = P 0.01

Matrix A : Control plants

1 Grain yield	1.000									
2 Biological yield	0.886	1.000								
3 Harvest index	0.584	0.204	1.000							
4 Days to anthesis	-0.267	0.031	-0.686	1.000						
5 Tillers	-0.537	-0.456	-0.485	0.426	1.000					
6 Height	-0.158	0.187	-0.651	0.771	0.442	1.000				
7 Average grain weight	0.593	0.699	0.300	-0.137	-0.696	-0.006	1.000			
8 Grains ear	0.892	0.708	0.568	-0.213	-0.253	-0.118	0.201	1.000		
9 Grain phosphorus (%)	-0.486	-0.309	-0.728	0.514	0.578	0.348	-0.594	-0.317	1.000	
10 Grain nitrogen (%)	-0.329	0.006	-0.802	0.454	0.324	0.430	-0.165	-0.362	0.729	1.000
	1	2	3	4	5	6	7	8	9	10

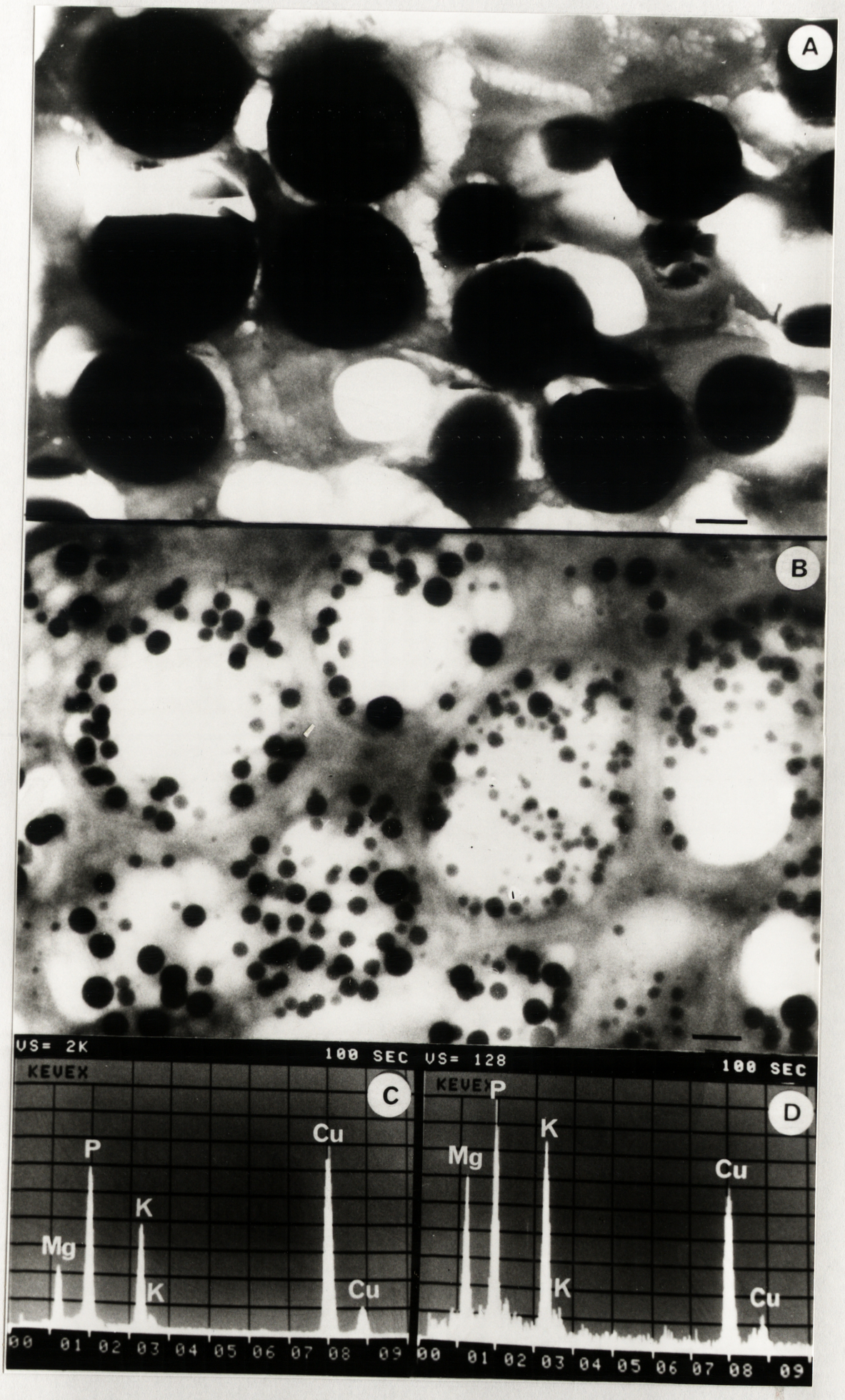
Matrix B : Low P plants

1 Grain yield	1.000										
2 Biological yield	0.861	1.000									
3 Harvest index	0.660	0.314	1.000								
4 Days to anthesis	-0.471	-0.273	-0.741	1.000							
5 Tillers	-0.610	-0.600	-0.645	0.672	1.000						
6 Flag leaf at anthesis(1)	0.382	0.320	0.413	-0.141	-0.189	1.000					
7 Height	-0.233	0.163	-0.587	0.507	0.166	-0.042	1.000				
8 Average grain weight	0.501	0.680	0.361	-0.420	-0.730	0.317	0.176	1.000			
9 Grain ear	0.925	0.695	0.717	-0.457	-0.491	0.382	-0.371	0.222	1.000		
10 Grain phosphorus(%P)	-0.625	-0.496	-0.815	0.744	0.748	-0.391	0.276	-0.605	-0.592	1.000	
11 Grain nitrogen (%N)	-0.409	-0.315	-0.566	0.604	0.541	-0.176	0.263	-0.512	-0.312	0.617	1.000
	1	2	3	4	5	6	7	8	9	10	11

(1) Proportion of area still green

PLATE 1. Transmission electron micrograph of a portion of aleurone cell from control (A) and low P (B) wheat grain (cv. Kite). Bar = 1 μm.

EDX analysis spectra of globoid crystals in aleurone cells of control (C) and low P grains (D). vs = vertical scale.


A
B
VS= 2K
100 SEC
KEVEX
C
Mg
P
K
K
Cu
Cu
00 01 02 03 04 05 06 07 08 09
VS= 128
100 SEC
KEVEX
D
Mg
P
K
K
Cu
Cu
00 01 02 03 04 05 06 07 08 09

CHAPTER 8

THE COMPARTMENTALIZATION OF PHOSPHORUS IN WHOLE, EXCISED WHEAT LEAVES

8.1 INTRODUCTION

The wheat plant with a low phosphorus supply grows slowly, is smaller overall and has a lower concentration of phosphorus, especially inorganic phosphorus, in all tissues (Chapter 4). By means of compensatory mechanisms, such as reduced tillering, reduced seed set, early senescence etc., the growth and yield of the plant is optimized.

For experimental purposes it would be useful to compare plants of similar size, grain number etc. which differ only in tissue phosphorus concentration. This would for example facilitate studies on photosynthesis in relation to cytoplasmic inorganic phosphorus levels (Chapter 1.3.3) and studies on the translocation of phosphorus in relation to carbon (Chapter 5). This chapter examines methods which appear to make possible short term manipulation of tissue phosphorus levels.

Several workers have reported enhanced rates of photosynthesis when intact plants or detached leaves were resupplied with inorganic phosphorus (Bouma, 1967; Nghia et al., 1981; Sawada et al., 1982) or some organic phosphorus compounds (Bouma, 1975). As Bouma (1967) stated it is not possible to distinguish direct from indirect effects of phosphorus on photosynthesis in such studies. An alternative approach is to grow control plants to the required stage of development then reduce the amount of phosphorus in the tissue by infusing into the plant compounds which react with phosphorus. For example, Loughman (1966) showed that mannose, iodoacetate, fluoride, arsenate and 2,4 dinitrophenol will reduce the transport of phosphorus from roots to shoots.

Professor David Walker and colleagues at Sheffield University , have examined the ability of D+ mannose to sequester cytoplasmic ortho phosphate | and in C_3 species this is believed to sequester inorganic phosphorus in the cytoplasm and reduce the export of triose phosphate out of the chloroplast and therefore starch accumulates in the chloroplast (Figure 8.1). Chen-She et al. (1975) showed that mannose stimulates the formation of starch in spinach beet (Beta vulgaris L.) and related species. In C_4 species regeneration of the CO_2-acceptor may be affected because mannose sequesters orthophosphate (Herold et al., 1976).

The rate of CO_2 uptake during photosynthesis of spinach beet and spinach (Spinacia oleracea L.) was reduced to about half following mannose infusion in the experiments reported by Walker and Herold (1977) and Harris et al. (1983) but the effects of mannose on photosynthesis, stomatal conductance and associated parameters in wheat leaves are not known and cannot be assumed from earlier studies. Herold et al. (1976) found that Beta vulgaris produced 1500% more starch but Hordeum vulgare only 300% more starch following a mannose treatment. Preliminary investigations with wheat revealed that mannose has a rapid and dramatic effect on photosynthesis so studies were conducted to determine if this technique could be used to manipulate cytoplasmic phosphorus levels in detached wheat leaves.

Studies of phosphorus in intact tissues would be aided if the amount of phosphorus in the cytoplasm and vacuole could be distinguished and measured. Several techniques have been used to examine vacuoles. Mesophyll cells of wheat leaves are too small to sample directly with micropipettes as used by Kirst and Bisson

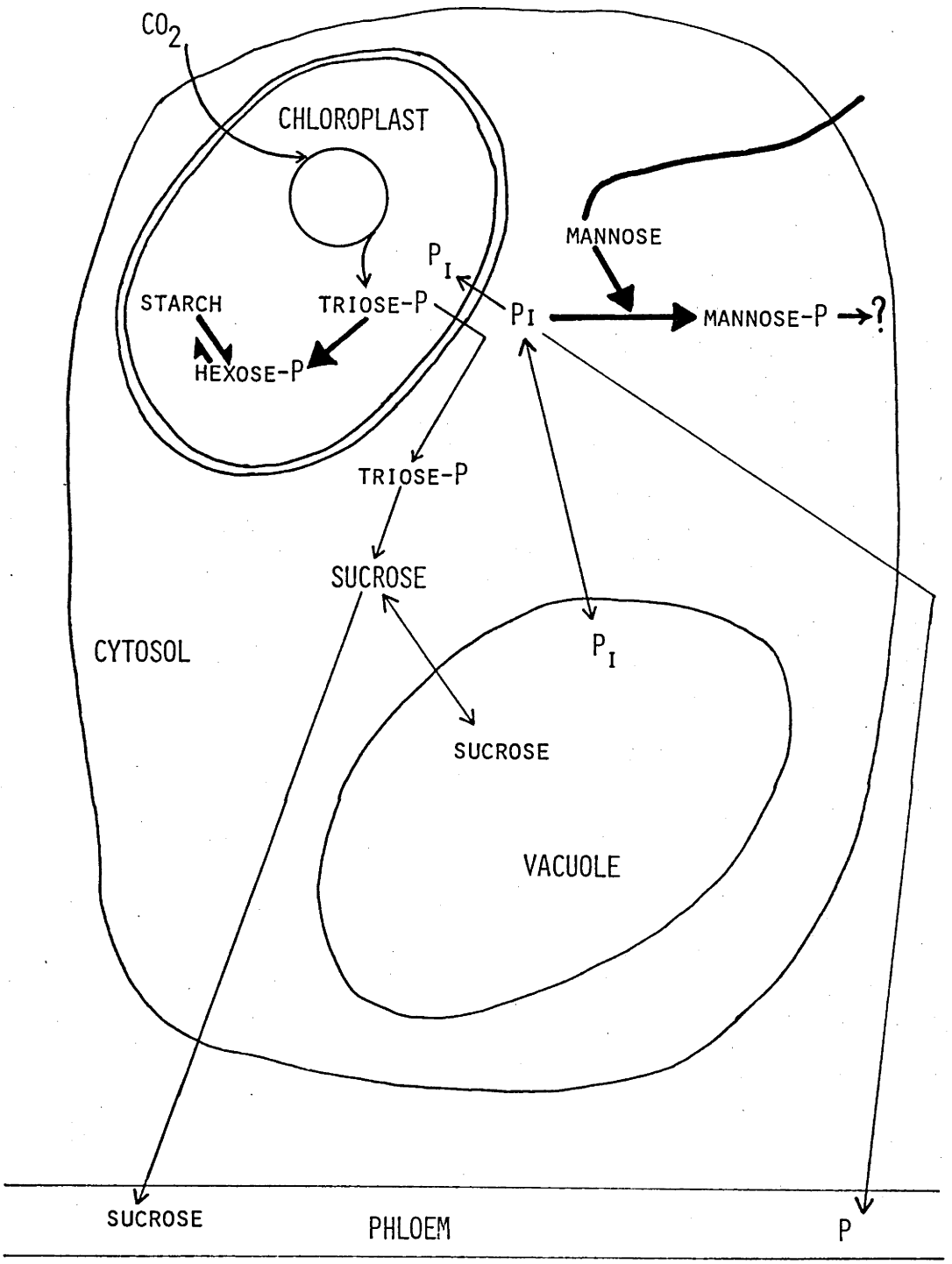


Figure 8.1 Effects of mannose on assimilation in mesophyll cells of C_3 species. Heavy lines indicates dominant reactions in the presence of D+ mannose - based on Chen-She et al. (1975).

(1982). Isolation of vacuoles via protoplasts from cell suspensions (Lörz et al., 1976) is not a suitable technique in many experiments because it is (of necessity) destructive of the tissue. Nuclear magnetic resonance (NMR) has been used to observe cytoplasmic and vacuolar phosphorus in corn root tips (Roberts et al., 1980; Kime et al., 1982a,b; Lee and Ratcliffe, 1983a), pea root tips (Lee and Ratcliffe, 1983b), spinach and asparagus cell preparations (Foyer et al., 1982) and potato tubers (Kime et al., 1982a,b,). This separation of inorganic phosphorus in the cytoplasm and vacuole by NMR is achieved by virtue of different pH's in these compartments.

Other species and other tissues have been examined superficially but further technical improvements are required to obtain clear resolution of vacuolar and cytoplasmic phosphorus (Kime et al., 1982a).

Differentiation of phosphorus in cytoplasmic and vacuolar compartments of cells in whole wheat leaves may be difficult due to the relatively low total phosphorus concentration (Kime et al., 1982a), the larger ratio of vacuolar to cytoplasmic volume (Komor et al., 1982) air spaces in the tissue (Lee and Ratcliffe, 1983a), and the presence of phosphorus in the apoplast, xylem and phloem at different pH's relative to the cytoplasm (Pate, 1976).

As part of this program an NMR technique was developed with wheat leaves which allows vacuolar phosphorus to be identified in contrast to phosphorus in the remainder of the tissue (intra and extra cellular).

8.2 MATERIALS AND METHODS

8.2.1 General

Plants were grown in sand as described in Chapter 2. Specific details are given in captions to tables or Figures below. Leaves were excised and immediately recut under water to prevent air entering the transpiration stream.

8.2.2 Photosynthesis studies in the mannose

A single, detached leaf with the base in water was placed in a portable leaf chamber (Parkinson et al., 1980) and allowed to establish a stable photosynthetic rate and steady stomatal aperture in an open ended gas exchange system similar to that described by Morison and Gifford (1983). The photosynthetic photon flux density at the leaf surface was 1100 μmol (quanta) $m^{-2}s^{-1}$ and the CO_2 concentration of the air entering the chamber was adjusted using mass flow controllers (models FC-260 and FC-261; Tylan Corp.). Using this system it was possible to accurately control and monitor the leaf temperature, the CO_2 and water vapor concentrations entering and leaving the leaf chamber. From these measurements conductance of water vapor via the stomata, net assimilation rate (= NCE) and leaf intercellular CO_2 concentrations were calculated using the relationships discussed by Von Caemmerer and Farquhar (1981).

8.2.1 NMR measurements

Excised leaves were transferred from a beaker containing water to one with buffer (10 mM Bicine - N,N-bis(2-hydroxyethyl)glycine- at pH 8.0) and placed in sunlight for 40 minutes. The leaves were quickly trimmed, rolled tightly, tied with fine cotton thread, and placed in a 10 mm diameter NMR tube. The sample was scanned

immediately in a Jeol FX90Q Fourier transform spectrometer operating at 36.2 MHz with a pulse repetition rate of 93 ms at a pulse angle of 45°. The sample was in the dark at ambient temperature (28°C) and rotated at 30 revolutions per second.

The fresh sample being scanned contained about 80 mg of dry matter. Compartment pH's were determined from the shift in the resonance (position of peaks on the spectra) relative to methylene diphosphonic acid (Figure 8.6).

8.3 RESULTS

8.3.1 Photosynthesis studies with mannose

Initial checks confirmed that infusion of sugars (L-mannose, D+ glucose) did not reduce photosynthesis and that the response to mannose was not an osmotic effect.

Mannose*, at a concentration of 1 mM, had only a slight effect on photosynthesis but within 15 minutes of beginning infusion with 10 mM there was a rapid and substantial decline in photosynthesis to less than half the steady, pre-mannose rate (Figure 8.2a). With continuous infusion of 10 mM D+ mannose photosynthesis remained at the new low rate, or increased slightly, before declining to below 20% of the initial level 80-90 minutes after beginning the infusion. At 10 mM 2-deoxglucose, which also sequesters inorganic phosphorus, had a similar effect to 10 mM D+ mannose (data not shown).

During the intial rapid decline in assimilation stomatal conductance did not fall to balance the increase in intercellular CO_2 and the ratio of intercellular:ambient CO_2 (the C_i/C_a ratio) increased (Figure 8.2b,c). Thus during this phase assimilation was not coupled

* unless stated mannose refers to D+ mannose

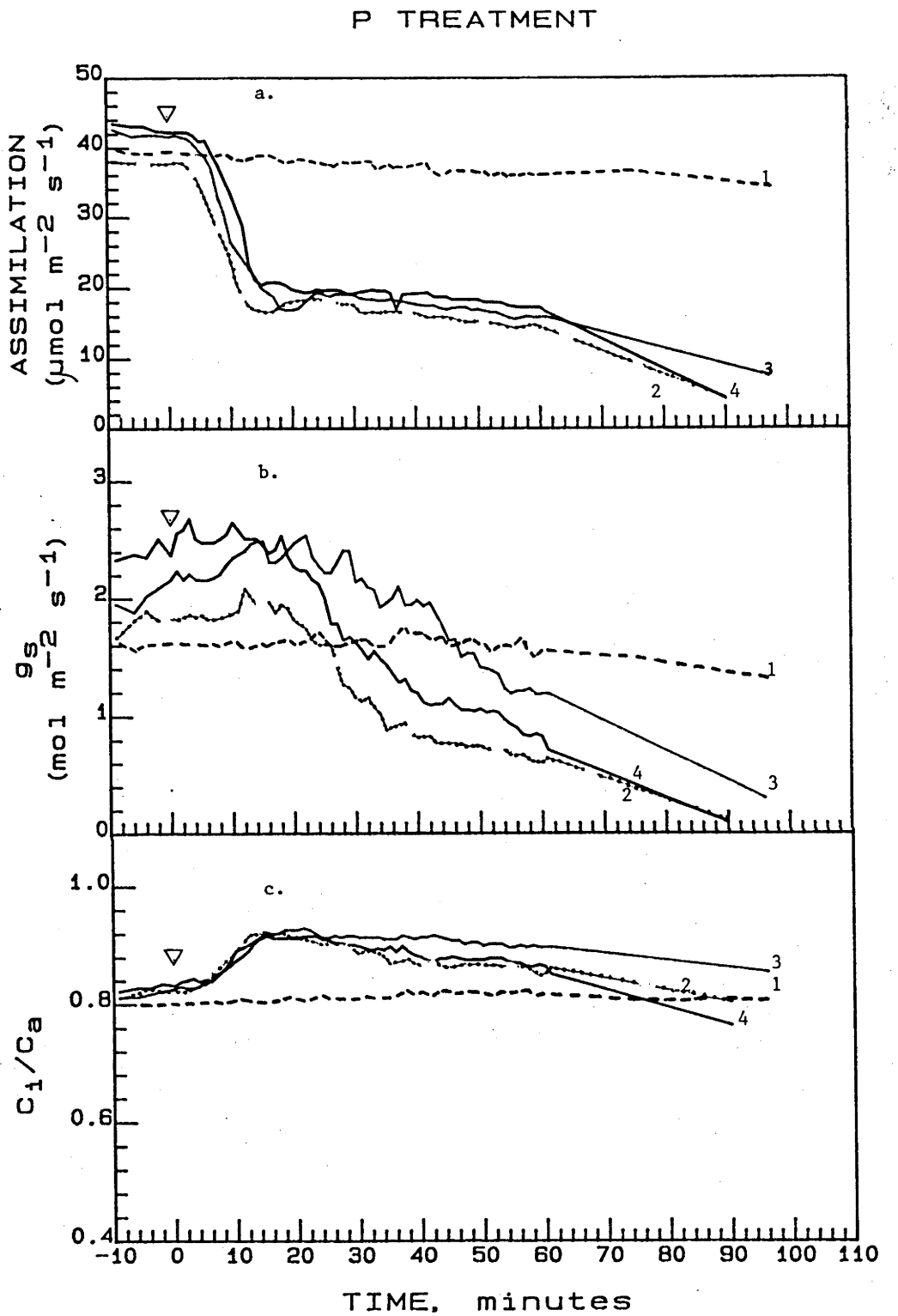


Figure 8.2 Effect of D+ mannose on (a) assimilation, (b) stomatal conductance (g_s) and (c) the intercellular:ambient CO_2 concentration. Lines were plotted from readings taken at 1 min intervals from chart recorder traces and are for flag leaves, of control plants or plants grown with nutrient solution which contained ¼, 1.0 and 4 times the control phosphorus supply throughout, infused with 10 mM mannose (2,3 and 4 respectively).
▽ indicates when mannose began to enter the transpiration stream.

to stomatal conductance via intercellular CO_2. After about 30 minutes stomatal conductance declined until, at about 90 minutes, the stomates were effectively closed. During the latter stages this closure caused the final drop in assimilation.

During these 90 minutes experiments leaves of plants from contrasting phosphorus regimes produced similar reactions to 10 mM mannose. The only noticable difference was that leaves with 0.1% total phosphorus had a lower rate of photosynthesis at 90 minutes than leaves with 0.3 or 0.5% total phosphorus (Figure 8.3).

Infusion of the product formed when D+ mannose is sequested in cells, i.e. mannose-6-phosphate at 10 mM into a fresh excised leaf caused a rapid and complete shut down in photosynthesis which was coupled to a decrease in stomatal conductance; hence there was a decline in the C_i/C_a ratio (Figure 8.4). This compound also appeared to directly reduce photosynthesis because the initial rate could not be recovered by raising increasing the concentration of CO_2 (C_a) around a leaf infused 1 mM with mannose-6-P.

8.3.2 NMR measurements

NMR spectra of whole excised wheat leaves are shown in Figure 8.5. Data for three thousand pulses, accumulated within 10 minutes of removing the leaves from the sun and buffer supply were sufficient to distinguish phosphorus in two compartments (i.e. with contrasting pH's). Maximum differentiation occurred after 17-20 minutes or 7000-9000 pulses (8.5b) but diminished quickly so that 10 minutes later peak 2 became indistinguishable from peak 3 (as in unbuffered leaves) due to broadening of the peaks. Using the titration curve in Figure 8.6 the pH of peak 2 was estimated to be 8.0 and peak 3

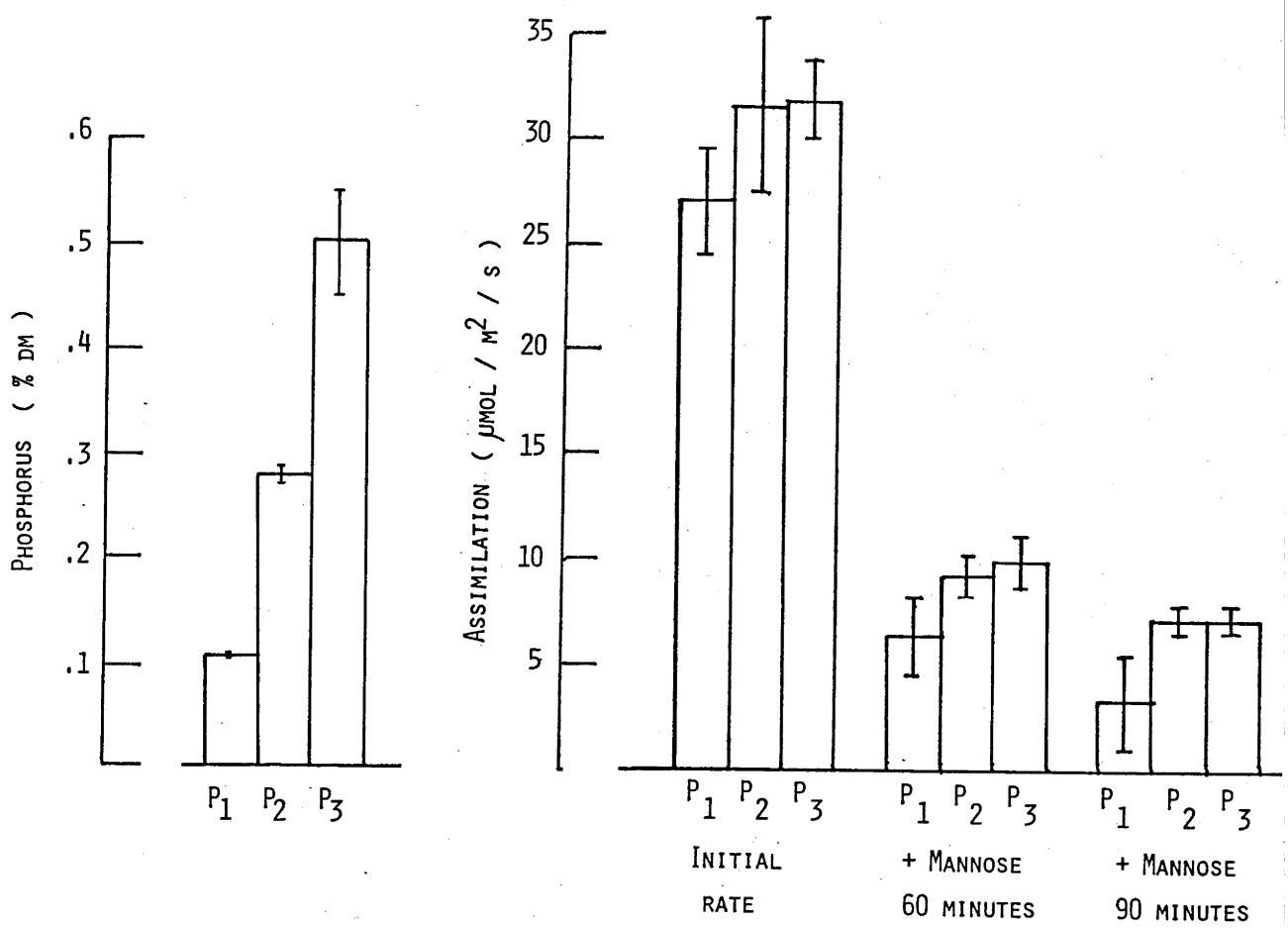


Figure 8.3 Total phosphorus concentrations, initial assimilation rate and the effect of D+ mannose* on assimilation after infusion for 60 and 90 minutes by plants grown with ¼ (P_1), 1.0 (P_2) and 4 (P_3) times the control rate of applied phosphorus throughout the growing season.

* 10 mM

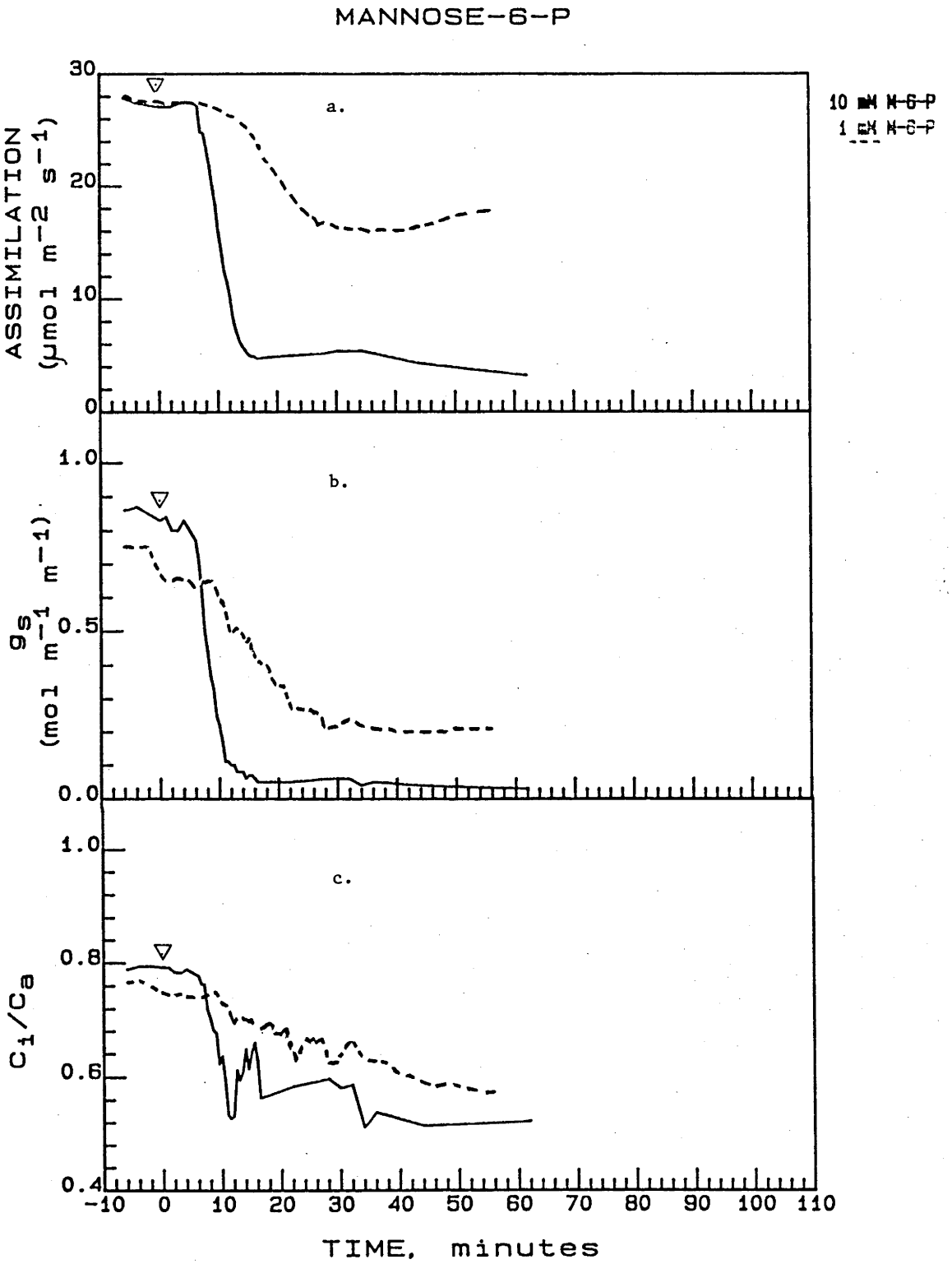


Figure 8.4 Effect of mannose-6-phosphate on (a) assimilation, (b) stomatal conductance (g_s) and (c) the $C_i:C_a$ ratio in the flag leaf of plants grown with the low P regime. -- 1 mM and —— 10 mM mannose-6-P.

pH 5.5. The shift difference between these two peaks was consistent from sample to sample. These findings are consistent with previous work and the peaks identified in Figure 8.5 are assigned. As follows: peak 1, glucose-6-phosphate; peak 2, inorganic phosphorus in the cytoplasm, apoplast and vascular system; and peak 3, vacuolar inorganic phosphorus.

No satisfactory spectra were obtained with leaves from plants grown with daily applications of nutrient solution which contained less than 1 mM P. The leaves used to obtain the spectra in Figure 8.5 contained a total of 4000 to 6000 μgP. gDW^{-1}.

8.4 DISCUSSION

8.4.1 Mannose

Two techniques were explored as possible means to examine directly the size and functions of cytoplasmic and vacuolar phosphorus in live, whole leaves.

Infusion of detached wheat leaves with D+ mannose produced rapid and dramatic decreases in photosynthesis. The initial reaction is consistent with sequestration of orthophosphorus in the cytoplasm and a reduction in the exchange of triose phosphate out of the chloroplast. Under the conditions of these experiments photosynthesis was reduced to less than half the steady rate within 15 minutes of mannose entering the transpiration stream. This is a more rapid and more severe effect than that reported by Harris et al. (1983) for spinach leaf discs infused with 50 mM mannose and not an osmotic effect as they suggested. The lack of an effect with 1 mM mannose indicates that wheat is able to metabolize mannose to sucrose as is barley (McCready and Hassid, 1941), or there is adequate

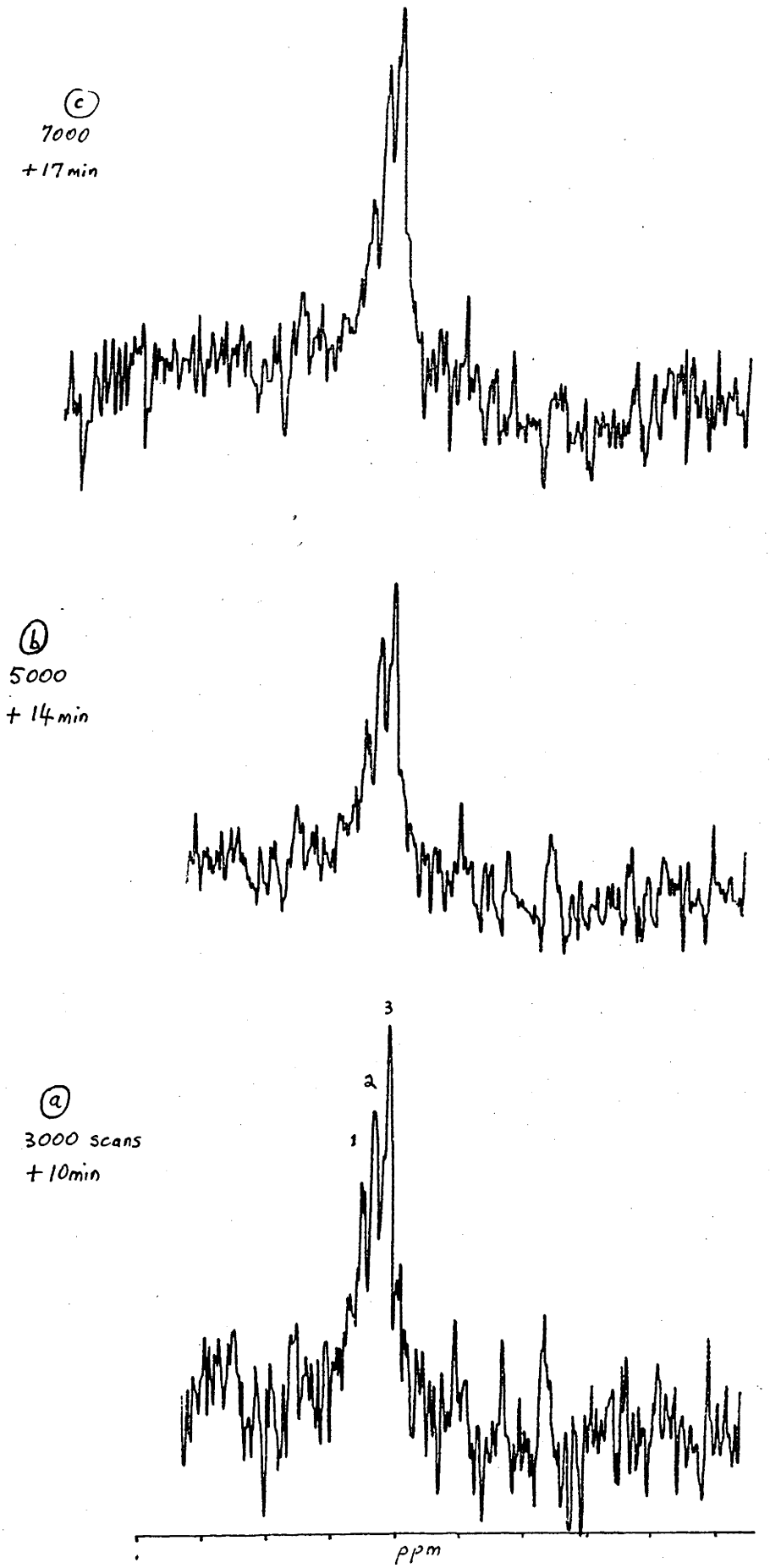
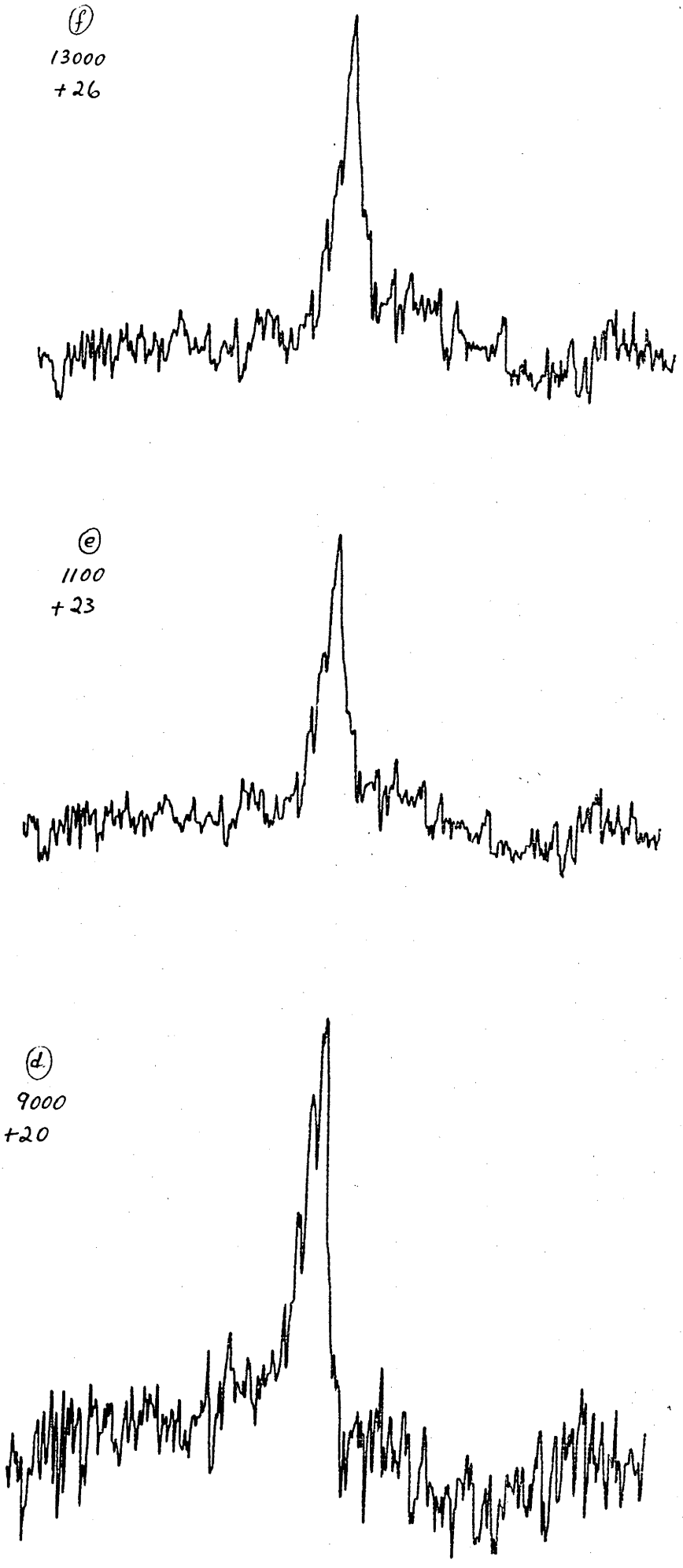


Figure 8.5 ^{31}P NMR spectra of fresh, whole wheat leaves from control plants infused with 10 mM Bicine buffer. Spectra were obtained progressively at 10 min or 3000 pulses (a) ---- 26 min or 13,000 pules (f) after the buffer treatment.


(f)
13000
+26
(e)
1100
+23
(d)
9000
+20

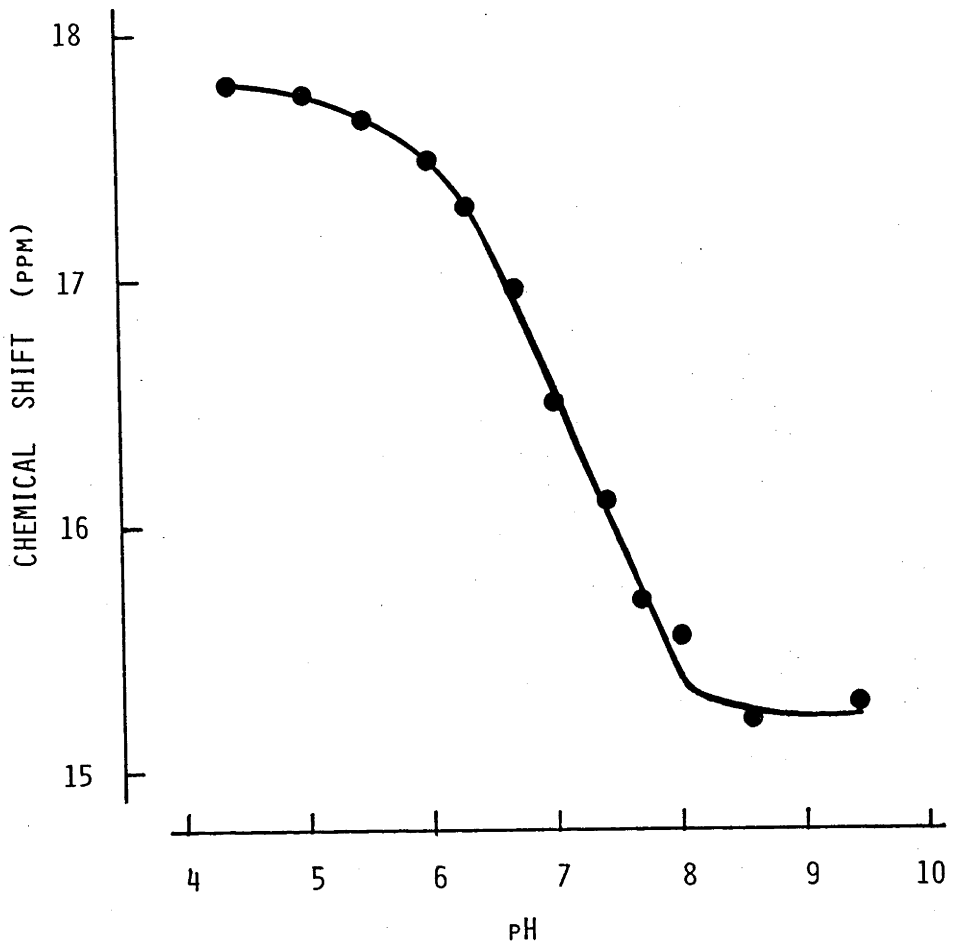


Figure 8.6 Relationship used to estimate the pH's of cellular compartments. The chemical shift refers to the position of the resonance peak for $^{31}P_i$ in buffer at pH's measured using a glass electrode assembly relative to the resonance peak for dimethyl phosphate.

exchange of phosphorus from the vacuole to replace that sequested by this concentration of mannose.

This study further showed that the product from sequestering phosphate with mannose, mannose-6-phosphate, is a powerful stomatal closing agent. Thus, in whole excised leaves, both sequestration of an essential ion and physical closure of the stomates reduced photosynthesis.

Fifty minutes after beginning the mannose infusion, uptake of mannose via the transpiration of water almost ceased due to stomatal closure. At this time the leaf was estimated to contain a total concentration of 1.5 $\frac{\text{m moles}}{l}$ of mannose and mannose-6-phosphate in an extra-vacuolar volume of 15.6 m moles of water. If the mannose/mannose-6-phosphate was uniformly distributed it would be at a concentration of about 0.11 M and have an osmotic potential of -0.32 MPa. (This figure obtained from relationships for pure mannose and mannose-6-phosphate compounds by Dr I.F. Wardlaw using a Wescor Thermocouple psychrometer). However, if transpiration via the stomates results in accumulation of solutes in cells adjoining the stomatal cavity it is possibly that the mannose/mannose-6-phosphate concentrated in that region of the leaf, and that the osmotic potential forced the guard cells of the stomates to lose turgor and close.

There was also some evidence that mannose-6-phosphate may have a direct effect on assimilation. This could be due to interference of mannose-6-phosphate with the action of the phosphorus translocator at the chloroplast membrane, or a feedback effect on export of triose phosphate due to additional hexose phosphate in the cytosol (Chapter 1, Figure 1.5).

It was interesting that plants with a large concentration of phosphorus did not offset sequestration of phosphorus by the infused mannose although at the 90 minute reading low P plants had the lowest rate of photosynthesis. Bieleski (1973) calculated that the half life of exchange of phosphorus between the vacuole and cytoplasm was in excess of 3 days. He quotes work for spinach which indicated that 14% of the phosphorus in the cell was in the cytoplasm, 14% in the chloroplast and 72% in the vacuole. Plants with higher total phosphorus also store phosphorus in organic compounds (Chapin and Bieleski, 1982; Chapter 4). Metabolism of these compounds could account for the slightly better rate of photosynthesis in high P plants following mannose infusion. However, if cytoplasmic phosphorus is as stable ($\mu g\ P\ g^{-1}$ FW) in leaves as in pea root tips (Lee and Ratcliffe, 1983b) then the increase in organic storage compounds noted above may be an artifact of the fractionation procedure.

The use of mannose to reduce cytoplasmic orthophosphorus appears to be of limited value for whole tissue studies because, it was not practical to infuse leaves with exogenous compounds via the transpiration stream after applying mannose. (There was negligible transpiration). Addition of mannose at a concentration between 1 and 10 mM may extend the period between the initial drop in photosynthesis and the secondary phase of the decline. However care would be required to ensure that side effects, due to mannose metabolism or mannose-6-phosphate, are not confounded with the desired effect (sequestration of phosphorus).

Other compounds, 2-deoxyglucose and glucosamine also sequester orthophosphate ions (Herold et al., 1976). In wheat leaves, 2-deoxyglucose had a similar effect to D+ mannose (data not

presented). A compound which sequesters phosphorus without causing side effects such as shown with mannose-6-phosphate is required before controlled manipulation of tissue phosphorus is possible for whole leaf physiological studies of phosphorus.

8.4.2 NMR studies

Two compartments of phosphorus at different pH were distinguished in a relatively modest (90 MHz) NMR spectrometer using a relatively small sample of mature leaves. However it was necessary to modify the pH of the extra-vacuolar tissue and obtain a spectrum without delay. Kime et al. (1982a) suggested that $> 10^{-4}$ M phosphorus is required to obtain a spectrum. Here in control tissues only about 1.5×10^{-5} mole inorganic phosphorus (480 µg inorganic phosphorus) was actually scanned, and this has been resolved into three compartments.

The total phosphorus in pea roots studied with a 300 Mz NMRby Lee and Ratcliffe (1983a) ranged from 200 to 400 µg inorganic P g^{-1} fresh weight. The leaves in the present work contained an estimated >600 µg P_i g^{-1} FW for control plants and <200 µg for low P plants.

The cytoplasm and vacuole and cells usually have pH's of 7.0 to 7.5 and 5.0 to 5.5. respectively (Smith and Raven, 1979). Using NMR Foyer et al. (1982) reported the pH in asparagus cells as 6.75 for cytoplasm and 5.7 for vacuole. Roberts et al. (1980) reported pH values of 7.05 to 7.15 for the cytoplasm and 5.5 for the vacuole of corn root tip cells. Under constant aeration, the cytoplasm of corn root tips has a slightly higher pH at 7.2 to 7.35 (Lee and Ratcliffe, 1983a). Roberts et al. (1982) discuss the uncertainities about pH's based on shifts in NMR spectra and suggest that absolute pH data by

NMR are accurate to within 0.2 pH unit. Relative pH changes which occur in a standard medium can be accurate to ± 0.02 pH unit.

For the sample scanned in Figure 8.4 peak 3 was larger than peak 2. This indicates that more than half of the inorganic phosphorus in the leaf was in the vacuole. Further analyses are required to quantify relationships between leaf age, phosphorus supply and the deposition of phosphorus in vacuoles.

The NMR technique developed here could be valuable at its present level of development to compare compartmentalization between species such as Deschampsia flexuosa, which accumulate inorganic phosphorus (Nassery, 1970), Banksia ornata which accumulates polyphosphate (Jeffrey, 1964) and lotus, which produces more dry matter than white clover at a low and a high phosphorus supply (Hart and Jessop, 1983).

8.5 CONCLUSION

At this level of research answers to questions about phosphorus in cellular compartments should be sought using several techniques. In the words of Matile (1982) ...."There is little hope that future work with isolated vacuoles will lead to an understanding of vacuolar functions. It would rather seem that a synthesis of results from direct and indirect analysis of compartmentation, morphological and biochemical approaches may eventually help to develop concepts of how the cytoplasm of plants cells uses the large vacuolar space for various purposes".

The study of whole tissue using NMR will be assisted by the use of larger probes (which hold more dry matter and allow for spinning the sample at various angles relative to the magnetic field) and more powerful spectrometers.

CHAPTER 9

INTEGRATING DISCUSSION

9.1 Introduction and summary of main findings

Phosphorus is a highly mobile plant nutrient, even in phosphorus deficient plants, and is readily translocated from older to younger organs, especially to actively growing tissue (Williams, 1948).

The studies reported in this thesis have examined the transfer of phosphorus from photosynthetically active tissues to the grain of wheat. Attention was focused mainly on the movement of phosphorus from the flag leaf, which is largely responsible for assimilating the carbon used for grain development, the deposition of phosphorus into the grain, and the consequences of this transfer, in a modern wheat (cv. Kite). This transfer of phosphorus leads to a reduction in the rate of photosynthesis of carbon by the tissues and accumulation of phosphorus in the grain, some as phytate.

The main findings, which are listed below and subsequently discussed in a wider context, support the hypothesis that modern wheats use phosphorus inefficiently. That is, the concentration of phosphorus in the grain is greater than is required for the development of the grain. The evidence obtained during the present studies which supports this view is as follows.

1) Phosphorus supplied after anthesis raised grain phosphorus concentration but did not enhance grain yield in plants growing in sand with daily applications of complete nutrient solution which contained either 1 or 0.25 mM P.

2) Low P plants in the present studies were grown with a diminishing supply of phosphorus. Compared to control plants (supplied with phosphorus daily to maturity) the low P plants were smaller, with a photosynthetic rate per unit leaf area dominated by

senescence. They produced fewer spikelets and set fewer grains which were smaller at maturity. The harvest index ratio was also lower. The low P plants were considered to be similar to plants grown in the field in Australia.

Positive features associated with low P plants, when compared to the controls, were the lower total and phytate phosphorus levels in grain and the higher ratio of deposition in grain of carbon:phosphorus especially during the early phase of grain development.

3) More phosphorus was taken up after anthesis and the grain of low P plants had a higher concentration of phosphorus when plants were subjected to high (30°/25°C) compared to low (15°/10°C) temperatures prior to anthesis (0.31 vs 0.15% P, respectively).

4) The rate and duration of grain development were lower in low P plants but raising grain phosphorus content failed to increase the rate or extend the duration of grain development. Foliar applications of phosphorus to the glumes raised grain phosphorus without increasing grain yield per ear. (Central spikelet grains were 6% heavier than controls but had a higher phosphorus concentration).

5) Phosphorus exported from the flag leaf was derived from structural (lipid, DNA, RNA), ester, and inorganic phosphorus compounds. When the total phosphorus concentration fell below 0.12 to 0.15% P, photosynthesis declined rapidly due to the export of nitrogenous compounds from the leaf. Foliar applications of phosphorus to the flag leaf extended the leaf area duration by up to 21% but failed to increase the accumulation of dry weight in the grain.

6) Grains appear to absorb phosphorus readily and store that not required for structure and metabolic compounds in the form of phytate. In low P plants phytate did not form in significant amounts until the grain maturation stage - possibly when there was import of phosphorus from senescing tissues such as glumes and the peduncle. Analysis of globoid crystal in the aleurone cells of mature grains indicated that phytate has a similar P, K, Mg composition in both control and low P plants.

Grains which do not form phytate are apparently not disadvantaged by the low concentration of phosphorus per se, because negligible responses were obtained to foliar applied phosphorus as noted in (4).

7) A limited sample of modern (hexaploid) wheats had a higher harvest index, and yield per main culm and lower grain phosphorus concentration than their progenitors (the diploid and tetraploid wheats) when grown with control or the low P nutrient.

8) Experiments on the effect of the phosphorus sequestering sugar, D+ mannose, confirmed that vacuolar phosphorus exchanges slowly with cytoplasmic phosphorus. This technique and ^{31}P nuclear magnetic resonance spectroscopy were found to be of limited value for whole leaf studies of photosynthesis in relation to phosphorus.

9.2 Increasing grain yield while reducing grain phosphorus concentration

Figure 9.1 is presented as a summary of the characters of wheat which could be manipulated, or selected for, in order to achieve more grain yield per unit of fertilizer phosphorus in the plant.

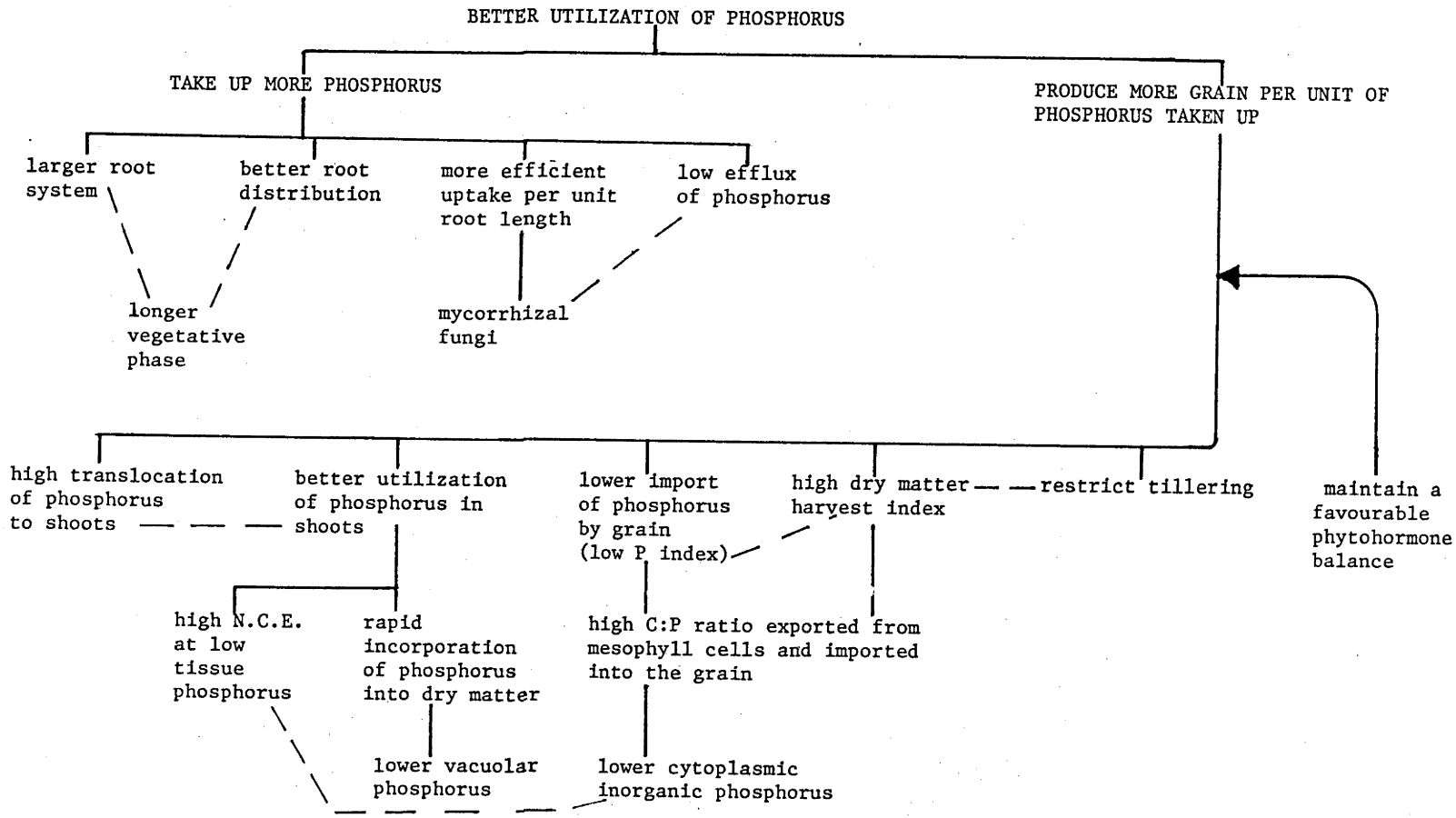


Figure 9.1 Some plant characters which, if modified, could lead to more efficient utilization of phosphorus by wheat. Dashed lines indicate interactions between characters.

9.2.1 The uptake of phosphorus

The value of early applications and uptake of phosphorus has been appreciated since the glasshouse studies of Gericke (1924) and Brenchley (1929) and field experiments by Smith (1967). In the first experiment reported in Chapter 3 there were strong interactions in plant phosphorus concentrations in old leaf and lower stem tissues, but there was no interaction in grain yield, between the amount of phosphorus applied per day and the period for which it was supplied. McLachlan (1982) obtained strong positive correlations between final grain yield and both the amount and the concentrations of phosphorus in shoots at the 4-5 leaf stage of wheat plants grown in the field. Phosphorus raises grain yields by increasing the number of spikes (tillers) m^{-2} and the number of spikelets ear^{-1}, i.e. grains m^{-2}, but in the field may cause grains to be lighter (Batten et al., 1984). Rahman and Wilson (1977) found that additional phosphorus caused similar increases in spikelet number in seven wheat genotypes by promoting the rate of spikelet initiation.

The promotion of tillers by early applications of phosphorus may in fact be a wasteful response by the wheat plant. In the field almost half of the tillers produced do not bear grain and higher yields have been obtained by reducing the number of tillers per plants (Borojevic and Kraljevic-Balalic, 1980; Islam and Sedgley, 1981). The optimum number of tillers m^{-2} or $plant^{-1}$ will vary with the environmental and the genotype (W.R.I., Boyd, personal communication).

The uniculm and oligoculm wheats reported by Atsmon and Jacobs (1977) should allow genetic control over tiller production but these wheats may be inefficient with respect to phosphorus. In Chapter 7 the oligoculm was found to have a high grain yield but a relatively high grain phosphorus concentration associated with a low harvest index. By breeding it may be possible to combine the high yield per culm of the oligoculm wheat with the high harvest index potential of semi dwarf wheats and thus reduce the grain phosphorus concentration.

The proportion of highly available fertilizer phosphorus "lost" to less available forms in the soil (Barrow, 1983, and references cited therein) would be reduced if plants absorbed phosphorus rapidly during the early stages of growth. Phosphorus uptake may vary with the root characteristics listed in Figure 9.1. Further studies are warranted on genotypic differences in the uptake of phosphorus by wheat (Schenk and Barber, 1980, predicted the uptake of phosphorus and potassium from root characteristics of corn).

Currently in Australia winter wheat genotypes are becoming available that are suitable for sowing in late summer when soil temperatures are warmer. The finding of Chien et al. (1982) suggest that fertilizer phosphorus may be more highly sorbed under the higher soil temperatures. Also, the plants have a longer vegetative period (prior to being vernalized) and are able to take up more phosphorus m^{-2} (Batten and Khan, unpublished data). Further field studies are required to determine the balance between these mechanisms and hence the actual phosphorus fertilizer requirements of wheats sown early in the season. The effects of temperature observed in experiment 2 of Chapter 3 suggest that both the current

and subsequent uptake of phosphorus can vary markedly with temperature.

Seedlings with the ability to take up phosphorus rapidly may be less prone to attack by pathogenic organisms.

Higher total, lipid and acid soluble phosphorus levels in the embryo of dry seeds, and in plants (older than 5-10 days) have been associated with resistance to rust (Tavadze and Budnitskaya, 1974; Tavadze et al., 1977). Thus early uptake of phosphorus may be important for healthy, as well as vigorous, plant growth.

This discussion will not focus on specific root characters associated with phosphorus uptake, nor on the potential role of vesicular-arbuscular mycorrhiza (VAM), however two points should be made here. VAM may be unnecessary, and may not infect plants which have access to adequate (e.g. fertilizer) phosphorus (especially under low light and at lower temperatures (Graham et al., 1982).

During the later phase of grain development 56% of the carbon exported from the flag leaf blade was directed to the roots and immature tillers (Table 5.5). If this carbohydrate stimulated the activity of VAM it could enhance the uptake of phosphorus during grain development and reduce the efficiency of utilization of phosphorus by simply raising grain phosphorus as discussed above or by stimulating the production of useless tillers. Or, if Allen et al. (1980) are correct, greater production of cytokinin type compounds in mycorrhizal plants may improve the phytohormone balance and allow significantly better plant growth (e.g. leaf longevity) and prolong grain development. Other interactions between host plants, VAM and other fungal organisms are discussed by Safir (1980).

9.2.2 The utilization of phosphorus within the plant

Over 80% of the grain dry weight is derived from the assimilation of carbon during the grain development phase and higher yields may be attained by increasing the rate and duration of photosynthesis (Austin, 1982). This appears to be particularly important in the low P plants because both the rate and duration of photosynthesis decline rapidly with the age of, i.e. the export of phosphorus from, the tissue. For example the flag leaf blade made no contribution to grain dry weight after the grains reached 60% of the final dry weight. To improve photosynthesis in low P plants either the rate of export of phosphorus from the structure and photosynthetic tissues must be reduced to prolong the leaf area duration, or the rate of photosynthesis at a given total phosphorus concentration in the leaf must increase.

The effect of tissue phosphorus on photosynthesis in wheat genotypes is not known but in Phaseolus vulgaris Whiteaker et al. (1976) found that photosynthesis per unit leaf area in one genotype was 41% higher than in another genotype when the leaf contained 0.28% phosphorus. A closer examination of the compartmentalization and the chemical forms (ester, lipid etc.) of phosphorus in genotypes with such differences may reveal if the more efficient genotypes require less phosphorus for metabolism or simply maintain less storage phosphorus. The chemical fractionation technique described by Bieleski (1968a), the rapid test for the inorganic phosphorus (Bouma and Dowling, 1982) and the NMR technique could all be useful in such studies.

To transfer less phosphorus out of photosynthetic tissues, without reducing translocation of assimilate requires differential loading of the phloem.

A proton gradient between companion cells and phloem sieve elements promotes the loading of sucrose into the phloem (Chapter 1.3). Dunlop et al. (1983) found that the entry of phosphorus into cultured cells of white clover petiole explants was at a maximum when the external medium was at a low pH (< 5). This work is consistent with the theory that an electrical potential and diffusion gradient is established across the cell wall by the existence of an ATP driven proton extrusion pump (Poole, 1978). This gradient facilitates the re-entry of protons into the cytoplasm and the re-entry of phosphate is coupled to that of protons.

If a proton motive force is used to load both sucrose and phosphate into the phloem then it may not be possible to increase sucrose loading without increasing phosphorus loading. At the mesophyll cell level efficiency may be gained by selecting for cells which maintain a low level of inorganic phosphorus in the cytoplasm.

But to maintain photosynthesis, at a lower inorganic phosphorus concentration in the cytoplasm, the efficiency of metabolism in the chloroplast (e.g. the reductive pentose phosphate pathway) and/or the efficiency of the phosphate translocator in the chloroplast membrane would need to increase.

An examination of the relative movement of phosphorus and sucrose in the phloem is required to determine if these move independently of each other, and if there is scope for screening genotypes according to the C:P ratio of phloem sap presented to the grain. Tapping of the phloem using aphid stylets may be a useful technique in such studies (Peel, 1975; D. Fisher, personal communication).

The utilization of phosphorus could also be improved if phosphorus was acquired actively, but only when needed, by the grain for structural and metabolic purposes.

The unloading of sucrose from the phloem is not understood and there is evidence for both passive and active processes (Gifford and Evans, 1981). The translocation studies in Chapter 5 show that carbon and phosphorus redistribution are somewhat independent. Under low phosphorus there was a lower export of phosphorus out of the leaves and a greater deposition in other non-grain tissues (especially the stems) than in control plants. This shows a compensation by the low P plant to retain phosphorus in vegetative tissues (i.e. in mobile or temporary storage forms) rather than in the grain where it is apparently of no value for grain growth (Chapter 6). It could be useful to compare the redistribution patterns of phosphorus in "efficient" and "inefficient" genotypes (e.g. wheat with high vs low phosphorus harvest indexes). Both Lipsett (1964) and Jessop et al. (1983) reported large genotype differences in harvest index for phosphorus in wheat.

It would also be useful to measure the contribution made by the glumes to grain dry weight as these tend to accumulate phosphorus exported from the flag leaf (Chapter 5) and remain green until the grains reach maturity. The tetraploid Polish wheat T. turgidum, conv. polonicum (No.21 in Chapter 7 data) is noted for having very large glumes and this wheat could also be valuable for such studies.

Because wheat cannot respond to a late supply of phosphorus, even in a controlled environment, but follows a pattern determined during an earlier stage of development, this adds support to the conclusion of Nooden and Leopold (1978) that factors other than

nutrient stress per se are primarily responsible for monocarpic senescence. It also suggests that the other factor(s) (the phytohormone balance) establishes control much earlier than previously believed. Michael and Beringer (1980) cite work which showed that nitrogen or potassium deficiency are associated with lower cytokinin production or translocation. Abscisic acid . . may influence phloem unloading and grain maturation. Further studies are required on the phytohormone balance in low P plants (see Letham et al., 1978).

9.2.3 Screening wheat for better phosphorus utilization

The small number of wheat genotypes compared in Chapter 7 showed that evolution (the increase in ploidy) has led to higher yields and to a lower concentration of phosphorus in grain in the main culm by virtue of an increase in harvest index. Plant breeding over the relatively short period of the last 100 years has also led to increases in yield and harvest index (Austin et al., 1980; England and G.M. Murray, Australia, personal communication).

There have been greater yield improvements in wheat than in other cereals during this century because wheat has been subjected to a heavier selection pressure. This could also account for the greater improvement in the yield of winter wheat in Sweden, 102 kg $ha^{-1}yr^{1}$, compared to 89 kg $ha^{-1}yr^{-1}$ for spring wheat (Mac Key, 1979).

These improvements are tangible evidence of the value of plant breeding and selection. Austin (1982) and Mac Key (1979) discussed breeding and physiological aspects of increasing yields, but no effort appear to have been given to selection for low phosphorus. Several earlier papers reported that grain phosphorus concentration is a genetically heritable trait (Rasmussen et al.;Peterson et al.,1983) but

the decline in grain phosphorus with increased yield or harvest index (Chapter 7) heritability figures may be confounded by variation in yield or harvest index. Nevertheless, at a given harvest index level there appears to be adequate variation between genotypes on which to exert selection pressure.

It should be possible to improve phosphorus utilization efficiency using two approaches:

- indirectly by selection and breeding for higher yield or harvest index
- directly by assessing the efficiency with which plants store, utilize and retranslocate phosphorus.

Breeding for higher yield may not lead to a lower grain phosphorus concentration if gains are achieved by increased uptake rather than by improved utilization of phosphorus by the plant. Thus, if yield or harvest index are improved then a check on grain phosphorus is still required as shown in Chapter 7 where line 28 had the highest yield, but not the lowest grain phosphorus.

9.3 Is a low grain phosphorus desirable?

(Advantages and disadvantages of reducing grain phosphorus)

The advantages have been discussed with respect to economics, the nutrient cycle and human nutrition in Chapter 1.

If the total phosphorus concentration in wheat is reduced there will also be a reduction in the proportion found in the form of phytate (Figures 1.12, 3.2). This is desirable for both economic and nutritional reasons.

For economic reasons there is increasing pressure to remove less bran when milling white flour and in many Western Societies a

consciousness about dietry fibre has encouraged more people to eat wholemeal products. Both changes have led to an increase in the consumption of phytate. Where the staple food is unleavened wholemeal cereals there is a great risk of nutrient deficiency if the total diet provides a marginal intake of minerals. This situation has occurred in such areas as Iran (Reinhold, 1971; Reinhold et al., 1983; Ter-sarkissian et al., 1974), Africa (Amoa and Muller, 1976), Mexico (Reinhold, personal communication), and even America (NAS/NRC, 1974) where phytate has been associated with low calcium, zinc and iron bioavailability, although there are discrepancies between workers as to the seriousness of the effects (Cheryan, 1980; Maga, 1982).

A reduction in grain phosphorus is likely to be associated with a reduction in grain protein because of the general negative relationship between yield and nutrient concentration under similar conditions (Chapter 7, Batten and Khan, unpublished field data). Within the bread wheats breaking the yield:N:P relation could be a barrier due to the importance of grain protein concentrations for bread making (Pomeranz, 1980). However, considerable gains in grain protein concentration, without a loss of protein quality for bread making, have been obtained by transferring genes from Triticum dicoccoides to bread wheats (A. Blum, personal communication; Law, 1982). Hopefully, the changes effected in protein concentration are not linked with increases in nutrients such as phosphorus. If nitrogen and phosphorus are closely linked then agronomic changes, such as short season genotypes, may result in sufficiently high protein while minimizing grain phosphorus.

Could grain with a low phosphorus content give poor germination or seedling vigour?

At present there is no evidence to answer this question. Either phosphorus levels have not been so low as to show any adverse effects; or the cultural practice of sowing seed with fertilizer masks the effects.

In wild oats (Avena fatua L.) Jain et al. (1982) found a negative correlation between inorganic phosphorus in the caryopsis and the relative depth of dormancy. Deeper dormancy is urgently needed to eliminate preharvest sprouting in wheats grown in areas with wet periods during grain development. Gordon (1979) discussed the possible roles of some pentose phosphorus compounds (and other grain compounds) in germination, but as King (1983) cautions, changes in dormancy may lead to an increase in activity of the pentose phosphate pathway rather than the inverse.

9.4 Conclusion

Clearly many aspects of low phosphorus wheat physiology and biochemistry remain to be studied. Hopefully some new techniques will assist the search for greater efficiency in the utilization of phosphorus by cereals.

In particular, this study suggests that the translocation of phosphorus relative to carbon in the phloem and the ability to continue photosynthesis at low tissue phosphorus concentrations warrant closer examination.

The ultimate test of a phosphorus efficient plant is that it is efficient when grown in the field. To date only a few field studies of phosphorus efficiency between genotypes have been published. If

more food is to be produced, especially in countries where fertilizers are not available or too expensive, further evaluations of genotypic variation are required.

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Wheat (cv KITE) growing at WAGGA WAGGA, N.S.W. AUSTRALIA.

Many men have strived to grow two blades of grass where only one grew before. It is now time to grow blades of grass and produce food using less fertilizer.

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APPENDICES

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APPENDIX 1: NUTRIENT SOLUTIONS

The nutrient solutions used in these studies were based on the Hoagland No. 2 solution (Hewitt, 1966) with modifications to the minor nutrients as used in the CSIRO, Division of Plant Industry (Canberra) Phytotron (CERES).

The phosphorus level in the nutrient solution was modified by substituting ammonium nitrate for ammonium dihydrogen phosphate (Table App. 1.1). The NH_4^+ : NO_3^- ratio varied as shown in Table App 1.2.

TABLE App. 1.1 Composition of Nutrient Solutions

Salt (mg L^{-1})		Element		
			mg L^{-1}	m mole
Phosphate treatments				
Control P				
$(NH_4)H_2PO_4$	120	P	32	1
Low P				
$(NH_4)H_2PO_4$	30	P	8	0.25
NH_4NO_3	31			
or				
P	nil	P	nil	nil
NH_4NO_3	41			

All solutions

$Ca(NO_3)_2.4H_2O$	950	N	212	15.1
KNO_3	610	K	236	6.0
$MgSO_4.7H_2O$	490	Ca	161	4.0
H_3BO_3	0.6	Mg	48	2.0
$ZnSO_4.7H_2O$	0.09	S	67	2.1
$CuSO_4.5H_2O$	0.05	Cl	0.143	µ mole
H_2MoO_4	0.02	Fe	5.0	260
$Co(NO_3)_2.6H_2O$	0.025	B	0.105	10
* $FeSO_4.7H_2O$	24.9	Co	0.005	0.085
* Disodium EDTA	33.2	Mo	0.012	0.125
* NaOH	5.0	Mn	0.111	2.0
		Cu	0.013	0.20
		Zn	0.020	0.31

* solution aerated overnight

(The pH of the solutions applied to the pots was adjusted to 6.5 with 1N NaOH solution).

TABLE App. 1.2. pH and nitrogen ratios in solutions with various phosphate levels (before final pH adjustment)

Solution P (ppm)	pH	NH_4^+ : NO_3^- ratio
32	5.1	1:13.5
8	5.7	1:21
nil	6.1	1:28

Likely effects of the change in the NH_4^+ : NO_3^- ratio

The growth and chemical composition of plants fed NO_3^- N differ from those fed NH_4^+ N (DeKock, 1970). Leyshon et al. (1980) noted that wheat plants fed only NO_3^- N were taller, had thicker stems and more spikelets per spike, but less spikes than in NH_4^+ N only treatments. The nitrogen sources produced plants with similar total dry matter at maturity but those fed NH_4^+ N produced more grain. Plants supplied with NH_4^+ N usually do not grow as well, contain lower concentrations of cations (e.g. Ca^{++}, Mg^{++} and K^+), whereas the elements originally absorbed as anions (e.g. $SO_4^=$, $PO_4^{\equiv}$ and Cl^-) are often found in higher concentrations than in plants supplied with NO_3^- N. Amino acids may be higher, but organic acids, lower in plants fed NH_4^+ N (DeKock, 1970; Kirkby and Hughes, 1970).

It is not possible to accurately assess the effects of the changes in the ratio of NH_4^+ : NO_3^- as used here with experiments reported in the literature. In most studies the plants were fed nitrogen in one or the other form only (e.g. Kirkby and Mengel, 1967; Blair et al., 1970; Leyshon et al., 1980; Barneix and Arnozis, 1980) or the ratios used were widely different to those used here (e.g. DeKock (1970) used NH_4^+ : NO_3^- ratios of 1.1:1 and 1:5.5). However, it is possible to suggest that the increase in NO_3^- N, relative to NH_4^+ N, in the low-P solutions used here would have tended to suppress the uptake of anions (e.g. $HPO_4^=$, $H_2PO_4^-$, $SO_4^=$, Cl^-) and enhance the uptake of cations (e.g. K^+, Ca^{++}, Mg^{++}, Na^+).

The anion:cation balance would be maintained (Kirkby and Mengel, 1967; Kirkby and Knight, 1977). The effects of nutrient deficiencies on the concentrations of elements in maize leaves are shown in Table App. 1.3 (from Clark, 1981).

Table App. 1.3.

- From Clark (1981)

Table 15 Mineral element concentrations of maize leaves of plants grown at low levels of mineral elements in nutrient solution (from 45)

Element deficiency	Dry matter yields (% of control)	Element concentration										
		N	P	K	Ca	Mg	S	Mn	Fe	B	Cu	Zn
		%						μg/g				
None[a]	100	3.35	1.26	4.95	0.62	0.37	0.30	39	118	20	14	62
NH_4^+-N	76	2.79	0.66	7.63	0.83	0.35	0.31	72	134	14	10	43
NO_3^--N	61	2.04	1.02	4.52	0.32	0.18	0.38	19	309	16	10	21
P	34	3.20	0.44	4.71	1.05	0.47	0.37	38	1968	37	21	72
K	20	4.06	2.85	1.25	2.24	0.77	0.49	92	585	51	15	92
Ca	30	3.94	3.18	6.84	0.05	0.32	0.32	81	1029	27	24	89
Mg	29	2.95	1.73	5.91	0.79	0.07	0.19	86	252	30	20	84
S	38	4.88	1.47	3.70	0.52	0.39	0.05	36	184	28	17	43
Mn	27	4.27	1.74	6.54	0.76	0.35	0.39	11	218	28	22	68
Fe	63	4.12	1.48	7.36	1.08	0.32	0.40	97	63	14	14	67
B	102	3.34	0.92	5.05	1.22	0.50	0.25	78	147	8	13	39
Cu	78	3.87	0.82	5.97	1.02	0.39	0.30	63	238	14	7	87
Zn	57	4.05	2.46	6.99	1.23	0.41	0.37	115	1813	33	21	19

[a]Separate nondeficient controls were grown with each element deficiency, because five levels of each nutrient deficiency were used. The values for the nondeficient leaves are those of the control for the element which was in deficiency: N values are for the NO_3^--N control.

APPENDIX 2 : POTTING SAND

Local river sand was steam sterilized, placed in woven PVC bags and surface sterilized with methyl bromide prior to use in the Phytotron.

After potting, loose particles of soil and water soluble salts were flushed from the sand with water. The sand was held in the free-draining pots by small squares of paper towel.

The sand had a bulk density of 1.13 g cc^{-1}, the particle size distribution shown in Table App. 2.1, and held 25% water at saturation (1 cm suction) and 2.9% water at field capacity (-10 Jkg^{-1}).

TABLE App. 2.1.

Size (mm):	<4	2-4	1-2	.5-1	.25-.5	.125-.25	<.125
% sample:	2.3	10.1	22.9	40.6	21.8	1.8	0.4

The sand was analysed for phosphorus by a fractionation procedure before and after the application of nutrient solution for 20 days at 24/19°C (Table App. 2.2.)

TABLE App. 2.2: Phosphorus in potting sand (ppm P).

Sample	Unused	Low P (0.25 mMP)[1]	Control (1.0mMP)[1]
Phosphorus fraction			
Total P[2]	28.9	36.3	57.7
Mineral P[2]	24.5	33.9	55.8
Organic P[3]	4.4	2.4	1.9
Easily soluble P[4]	0.9	1.7	6.6
Labile P[5]	1.1	5.2	13.8
Organic P[5]	3.2	5.9	10.5
Acid soluble P[5]	11.5	11.8	12.9

1: P in nutrient solution (see Appendix 1).

2: Ignition method, 400°C for 6 hours (Hedley et al., 1982).

3: by difference between total and mineral P.

4: 1.0 M NH_4Cl (Chang and Jackson, 1957).

5: 0.5 M $NaHCO_3$, pH 8.5; 0.1 M NaOH; and 1.0 M HCl respectively (Hedley et al., 1982).

These four pools were determined on 2 g samples extracted successively by overnight shaking at room temperature.

APPENDIX 3: AN EXAMINATION OF EXPERIMENTAL VARIANCE

A population of 80 wheat plants (cv. Kite) was grown at 24°/19°C in an open glasshouse in Autumn 1981. Single plants in 1 kg of sand in 127 mm pots were supplied with nutrient solution containing 0.25 mM P each morning and demineralized water each afternoon to maturity. After senescence of the tillers (those which had formed ears before the main culm reached anthesis) each plant was divided into shoot and root. The main culm was examined in more detail and the grain analysed for total phosphorus and nitrogen.

Data from the plants in this population were used to obtain estimates of the mean ($\bar{X}$), standard deviation (s) and the standard error of the mean (sem) for different numbers of plants (n). The number of replicates (n) needed to obtain accurate treatment means in future experiments, were calculated as:

$$n = \{t.s/(\bar{X}-m)\}$$

and where t = tabular t value and $\bar{X}-m$ = difference between treatments. Some of the n values calculated are shown in Table App. 3.1.

TABLE App. 3.1: Smallest detectable differences (sdd) between means in relation to the number of replicates.

Trait	mean	sdd		
	n = 80	5	10	30
Height (cm)	60.8	3.6	2.1	1.6
Tiller No.				
mature	3.58	0.51	0.40	0.23
immature	2.3	1.49	0.74	0.42
Spikelets				
fertile	14.9	1.0	0.89	0.59
infertile	0.79	0.63	0.38	0.22
Days to anthesis	43	2.2	1.0	0.6
Dry weight (g)				
total shoot	8.67	2.20	1.52	0.82
main culm				
shoot	2.56	0.24	0.17	0.14
flag leaf	0.208	0.032	0.026	0.015
grain	1.09	0.13	0.09	0.06
mg/kernel	31.3	4.1	2.7	1.7
grain (%P)	0.397	0.077	0.045	0.018
grain (%N)	3.688	0.206	0.131	0.102

APPENDIX 4: NOTES ON POTENTIALLY DANGEROUS CHEMICALS USED IN THE STUDIES REPORTED IN THIS THESIS.

This list is appended in the interest of safer laboratory practice. There is a concern that many research workers follow published methods without checking the potential danger of some reagents. This is not to say that methods which depend on toxic chemicals should be avoided. Rather, with an understanding of the hazards, chemicals should be handled following the appropriate precautions (e.g. gloves, masks, glasses, fume hoods, shielding etc.). Even storage of certain chemicals is hazardous. Careful labelling of all solutions etc. and removal from the work area of unnecessary toxic substances is also to be encouraged.

The notes which follow on specific compounds used in this thesis are based on information in The Merck Index (1976).

* Barium carbonate, $BaCO_3$

Danger: Poisonous, excessive salivation, vomiting, diarrohea, tremors, increased blood pressure etc.

LD_{50} (rats): 800 mg/kg

* Bayleton®, Methyl N-[1-butylcarbamoyl)-benzimidazol-2-yl]carbamate.

Other names: Benomyl; [1-[(Butylamino)carbonyl]-1H-benzimidazol-2-yl] acid methyl ester.

Danger: May cause skin irritations.

(Information from chemical company).

* ^{14}C, radioactive isotope of carbon.

Danger: Low energy β-particle emitter with half life of 5730 years. E_{max} = 0.156 MeV.
Maximum range in air 24 cm.

Notes: see ^{32}P.

* Hydrogen peroxide, H_2O_2 (30% w/w).

Other name: Hydrogen dioxide.

Danger: Strong oxidizing agent caustic to the skin and mucous membranes. Decomposed by many organic solvents.

Note: More stable in presence of mineral acid. Wear gloves. Store below 30°C.

* p-Nitrophenol, $C_6H_5NO_3$

Danger: Toxic. LD50 : 467 and 616 mg/kg; orally in mice and rats.

Notes: Wear gloves.

* Methyl bromide, CH_3Br

Other names: Bromomethane, monobromomethane, Embafume.

Danger: Toxic, odorless.

Lethal concentration: 514 ppm for rats in air (6 hours). High concentrations can produce fatal pulmonary edema, also narcosis. Chronic exposure can cause central nervous system depression and kidney injury.

Notes: Use only with approved safety equipment.

* Omite®, 2-(4-tert-butylphenoxy)-cyclohexyl prop-2-ynyl sulphite.

Other name: Propargite; Cyclosulfyne.

Danger: LD50 2,200 mg/kg in rats. Dangerous if swallowed, inhaled or absorbed.

Notes: Avoid contact with skin and inhaling vapor or spray. Use gloves and shield face, when handling concentrate, wash exposed parts of body after spraying. If swallowed induce vomiting (with ipecac). Do not give oily material.

(Information from chemical company).

* ^{32}P, radioactive isotope of phosphorus.

Danger: High energy β-particle emitter. Emax = 1.71 MeV.
Maximum range in air 720 cm.

Notes: Consult with local safety officer. Use in approved work areas. Label radioactive materials and equipment. Dispose of correctly.

Less than 1% of activity remains after 7 half life periods have elapsed. (i.e. 7 x 14.3 days).

* Perchloric acid, $ClHO_4$ ($HClO_4$) 70%

Danger: Very caustic, may deflagrate in contact with oxidizable substances.

Notes: Risk of explosion in digestions is reduced by mixing with H_2SO_4.

* P.P.O.

Danger: Phenol compound of undefined effects.

* Selenium, Se (powder)

Danger: Occupational exposure has caused pallor, nervousness, depression, garlic odor of breath and sweat. G.I. disturbances, dermatitis.

* Soda lime (5-20% sodium hydroxide, 6-18% water, and lime).

Danger: Strongly corrosive and irritation to skin, mucous membranes, eyes. Ingestion can cause severe damage to G.I. tract, death.

* Sulphuric acid, H_2SO_4 (conc.)

Other name: Oil of vitriol.

Danger: Very corrosive to all body tissues. Avoid contact with skin, do not inhale vapor.

Notes: When diluting, the acid should be added to the diluent. Keep tightly closed. Frequent skin contact with dilute solutions may cause dermatitis.

Wear gloves. Exhaust vapor using an effective fume hood.

* Toluene, C_7H_8

Other names: Phenylmethane, methacide, methylbenzene.

Danger: May cause mild macrocytic anaemia.

LD50 (rats): 7.53 ml/kg.

* Trichloroacetic acid, $C_2HCl_3O_2$

Other name: T.C.A.

Danger: Very corrosive.

LD50 : 3.3 g/kg in rats.

Notes: Keep tightly closed in a cool place. Storage of TCA solutions, in water, of less than 30% is not recommended.

Wear gloves. Irrigate affected parts with sodium carbonate solution.

APPENDIX 5: CRITICAL CONCENTRATIONS OF PHOSPHORUS IN CEREALS

The critical concentration of phosphorus for maximum yield of cereals is reduced with tissue age (Boatwright and Haas, 1961; McLachlan, 1982). In the standard reference manual on critical nutrient levels (Homer D. Chapman, 1966) whole shoot phosphorus concentrations are quoted as low, intermediate or high for plant growth. These categories are for whole shoots and the relative yield levels are not indicated (see Table APP 5.1A).

The approach of Gorshkova (1978) is somewhat more informative (Table APP 5.2). Phosphorus levels for barley, reported in the same paper, were slightly higher than for wheat at equivalent yield levels.

The tables APP 5.1 and 5.2 are included to give a general guide to phosphorus levels in tissues. Recently Bouma and Dowling (1982), Bouma (unpublished data) and Elliot et al. (unpublished data) have examined extractable (inorganic phosphorus) as a more reliable test of the phosphorus status of plants.

TABLE APP 5.1 Critical phosphorus levels in cereals (%P)

A. FIELD DATA

Species	Tissue age	Deficient	Low	Interm.	High	Toxic	Ref.
Wheat	heading	<.15	.15-.19	.20-.50	>.50		1
	straw		.03	.08	.17		2
	grain		.15	.40	.54		2
	grain	.39		.46			3
Barley	straw		.04	.12	.56		2
	grain		.15	.41	.62		2
	grain	.32		.44			3

B. CONTROLLED ENVIRONMENT STUDIES

Wheat	8 days					1.6	4a
	19 days					1.0	4a
	necrotic tip					to 4.9	4b

1. Ward et al. (1973);

2. Beeson (1941);

3. Greaves and Pittman (1946);

4a and b. Bhatti and Loneragan (1970a and b);

(2 and 3 cited by Chapman, 1966).

TABLE APP 5.2: Relationships between yield level and %P in wheat (Gorshkova, 1978)

Yield (% optimum)	Tillering	Shooting	Anthesis
<25	<.25	<.21	<.17
<40	.25-.28	.21-.23	.17-.18
40-70	.29-.31	.24-.28	.19-.22
<100	.32-.34	.29-.31	.23-.24
100	.35-.49	.32-.40	.25-.34
100 or less	>.49	>.40	>.34

APPENDIX 6: THE DISTRIBUTION OF PHOSPHORUS WITHIN PLANTS: DATA FOR CONTROL AND LOW P PLANTS IN CHAPTER 3

Data from Experiment 1

Table 6.1 Intraplant distribution of dry matter at anthesis (g ± sem)

Table 6.2 Intraplant distribution of dry weight at maturity (g ± sem)

Table 6.3 Intraplant phosphorus concentrations at anthesis (%P ± sem; n = 4)

Table 6.4 Intraplant phosphorus concentrations at maturity (%P ± sem; n = 3)

Table 6.5 Intraplant phosphorus at anthesis (mg P; n = 4)

Table 6.6 Intraplant phosphorus at maturity (mg P; n = 3)

Table 6.7 Intraplant nitrogen concentrations at anthesis (% N ± sem; n = 4)

Table 6.8 Intraplant nitrogen concentrations at maturity (%N ± sem; n = 3)

Data from Experiment 2

Table 6.9 Intraplant distribution at anthesis of dry weight, and phosphorus in low P plants following four preanthesis temperature regimes (n = 3 plants).

Table 6.10 Intraplant distribution at maturity of dry weight and phosphorus in low P plants following four preanthesis temperature regimes and grain development at 18/13°C (n = 3 plants).

TABLE App 6.1: Intraplant distribution of dry matter at anthesis (g ± sem)

TILLER	1 (main culm)	2	3	4	Total
a. CONTROL[1]					
Inflorescence	0.348(.015)	0.293(.036)	0.236(.019)	0.098(.015)	0.975
Flag leaf[2]	0.268(.017)				
Other leaves	0.314(.027)	0.510(.046)	0.498(.048)	0.275(.036)	1.865
Peduncle[2]	0.128(.007)				
Stem	0.309(.029)	0.296(.038)	0.237(.021)	0.101(.015)	1.071
Other[3]	0.408(.140)				0.408
Root-crown	0.707(.057)				0.707
Tiller DW	1.367	1.099	0.971	0.474	4.319
Tiller DW:Total shoot DW (%)	32	25	22	11	
Shoot:root					6.1
b. LOW P[1]					
Infloresence	0.343(.038)	0.149(.017)	0.026[4]		0.518
Flag leaf	0.232(.016)				
Other leaves	0.257(.022)	0.279(.024)	0.052		0.820
Peduncle	0.182(.011)				
Stem	0.388(.038)	0.202(.038)	0.033		0.805
Other	0.110(.019)				0.111
Root-crown	0.506(.032)				0.506
Tiller DW	1.402	0.630	0.111		2.253
Tiller DW:Total Shoot DW (%)	62	28	5		
Shoot:Root					4.5

1 for details see Table 3.1
2 only separated in main culm
3 late tillers
4 not all plants had 3 tillers.

Table App. 6.2 : Intraplant distribution of dry weight at maturity (g ± s.e.m.)

Tiller	1	2	3	4	5	Total
a. Control						
Grain D.W.	1.127(.122)	1.141(.019)	1.200(.133)	0.629(.221)	0.494(.236)	4.591
Grains ear^{-1}	32(3)	32(1)	33(4)	18(5)	13(4)	
Chaff	0.451(.025)	0.432(.040)	0.401(.046)	0.355(.039)	0.195(.021)	1.834
Flag leaf	0.238(.024)					
Other leaves	0.312(.015)	0.450(.010)	0.408(.022)	0.371(.010)	0.229(.053)	2.008
Peduncle	0.198(.038)					
Stem	0.367(.056)	0.479(.014)	0.452(.031)	0.400(.008)	0.255(.103)	2.151
Root-crown	1.232(.054)					
Tiller DW	2.693	2.502	2.461	1.755	1.173	10.584
Harvest index (%)	42	46	49	36	42	43
Shoot:root						9.1
b. Low P						
Grain DW	0.487(.078)	0.327(.048)				0.814
Grains ear^{-1}	20(4)	12(1)				
Chaff	0.341(.055)	0.199(.036)				0.540
Flag leaf	0.173(.022)					
Other leaves	0.211(.007)	0.230(.024)				0.614
Peduncle	0.195(.025)					
Stem	0.325(.032)	0.326(.080)				0.846
Root-crown	0.613(.034)					0.613
Tiller DW	1.732	1.082				
Harvest index (%)	28	30				
Shoot:root						4.6

TABLE App. 6.3: Intraplant phosphorus concentrations at anthesis (%P ± s.e.m.; n = 4)

Tiller	1	2	3	4
a. Control				
Inflorescence	0.385(.011)	0.455(.016)	0.471(.012)	0.621(.036)
Flag leaf	0.361(.025)			
Other leaves	0.348(.018)	0.425(.008)	0.426(.013)	0.470(.014)
Peduncle	0.453(.017)			
Stem	0.425(.021)	0.506(.022)	0.523(.014)	0.615(.041)
Other	0.571(.054)			
Root-crown	0.355(.029)			
b. Low P				
Inflorescence	0.166(.006)	0.169(.009)	0.140+	
Flag leaf	0.121(.003)			
Other leaves	0.079(.011)	0.137(.006)	0.130	
Peduncle	0.114(.012)			
Stem	0.046(.003)	0.100(.002)	0.112	
Other	0.069(.008)			
Root-crown	0.077(.004)			

+ not all plants had 3 tillers

TABLE App 6.4: Intraplant phosphorus concentration at maturity (%P ± s.e.m.; n = 3)

Tiller	1	2	3	4	5
a. Control					
Grain	0.596(.026)	0.579(.030)	0.598(.001)	0.620(.030)	0.613(.025)
Chaff	0.586(.066)	0.577(.055)	0.532(.080)	0.610(.014)	0.629(.018)
Flag leaf	0.147(.034)				
Other leaves	0.077(.014)	0.097(.017)	0.102(.014)	0.130(.024)	0.146(.070)
Peduncle	0.260(.062)				
Stem	0.036(.003)	0.162(.038)	0.145(.039)	0.160(.012)	0.149(.055)
Root	0.171				
b. Low P					
Grain	0.250(.024)	0.290(.029)			
Chaff	0.062(.016)	0.070(.011)			
Flag leaf	0.059(.004)				
Other leaves	0.035(.003)	0.036(.001)			
Peduncle	0.120(.004)				
Stem	0.035(.003)	0.038(.001)			
Root	0.062(.003)				

TABLE App.6.5: Intraplant phosphorus at anthesis (mg P; n = 4)

Tiller	1	2	3	4
a. Control				
Inflorescence	1.340	1.333	1.111	0.609
Flag leaf	0.967			
Other leaves	1.093	2.168	2.121	1.293
Peduncle	0.579			
Stem	1.313	1.498	1.240	0.621
Other	2.330			
Root-crown	2.510			
P in tiller	5.292	5.000	4.472	2.523
P in whole plant	22.126			
b. Low P				
Inflorescence	0.569	0.252	0.036	
Flag leaf	0.281			
Other leaves	0.203	0.382	0.068	
Peduncle	0.207			
Stem	0.178	0.202	0.037	
Other	0.078			
Root-crown	0.390			
P in tiller	1.438	0.836	0.141	
P in whole plant	2.881			

TABLE App.6.6: Intraplant phosphorus at maturity (mg P; n = 3)

Tiller	1	2	3	4	5
a. Control					
Grain	6.717	6.812	7.176	3.900	3.028
Chaff	2.643	2.493	2.133	2.166	1.227
Flag leaf	0.350				
Other leaves	0.240	0.437	0.416	0.482	0.332
Peduncle	0.515				
Stem	0.132	0.776	0.665	0.640	0.380
Root-crown	2.107				
P in tiller	10.597	10.518	10.380	7.188	4.969
P in whole plant	45.760				
b. Low P					
Grain	1.218	0.948			
Chaff	0.211	0.139			
Flag leaf	0.102				
Other leaves	0.074	0.082			
Peduncle	0.234				
Stem	0.114	0.124			
Root-crown	0.380				
P in tiller	1.953	1.293			
P in whole plant	3.626				

TABLE App.6.7: Intraplant nitrogen concentrations at anthesis (%N ± s.e.m.; n = 4)

Tiller	1	2	3	4
a. Control				
Inflorescence	2.079(.030)	2.357(.087)	2.458(.064)	2.715(.205)
Flag leaf	3.644(.144)			
Other leaves	3.690(.194)	3.414(.199)	3.339(.173)	3.019(.076)
Peduncle	2.119(.083)			
Stem	1.865(.101)	2.089(.087)	2.014(.079)	2.100(.132)
Other	3.382(.148)			
Root-crown	1.992(.151)			
b. Low P				
Inflorescence	1.789(.012)	1.838(.075)	1.685[+]	
Flag leaf	3.093(.080)			
Other leaves	2.723(.204)	2.725(.142)	2.703	
Peduncle	1.612(.039)			
Stem	1.680(.112)	1.874(.048)	1.669	
Other	1.971(.124)			
Root-crown	2.003(.127)			

[+] not all plants had 3 tillers

TABLE App. 6.8: Intraplant nitrogen concentrations at maturity (%N ± s.e.m.; n = 3)

Tiller	1	2	3	4	5
a. Control					
Grain	3.942(.156)	3.841(.030)	3.622(.094)	3.622(.249)	3.630(.367)
Chaff	1.554(.057)	1.569(.166)	1.324(.049)	1.597(.203)	1.604(.237)
Flag leaf	0.850(.066)				
Other leaves	0.893(.090)	0.795(.038)	0.707(.094)	1.060(.075)	1.087(.280)
Peduncle	0.648(.081)				
Stem	0.721(.155)	0.721(.100)	1.367(.025)	0.850(.043)	0.829(.108)
Root-crown	1.382(.118)				
b. Low P					
Grain	3.780(.225)	3.723(.137)			
Chaff	1.411(.277)	1.683(.226)			
Flag leaf	1.295(.014)				
Other leaves	1.396(.087)	1.367(.025)			
Peduncle	1.563(.101)				
Stem	0.764(.050)	1.066(.050)			
Root-crown	1.411(.132)				

Table App. 6.9 Intraplant distribution at anthesis of dry weight, and phosphorus in low P plants following four preanthesis temperature regimes (n = 3 plants).

a) Preanthesis temperature = 15/10°C

TILLER:	1 (Main culm)			2			Other		
	DW(g)	%P	mgP	DW	%P	mgP	DW	%P	mgP
Inflorescence	.333	.125	.416						
Flag leaf-blade	.195	.084	.164						
-sheath	.217	.068	.148						
Other leaves	.407	.053	.216	.458	.078	.357	.164	.087	.143
Peduncle	.186	.139	.259						
Stem	.663	.033	.219	.183	.148	.271			
Root	.885	.053	.469						

(b) Preanthesis temperature = 18/13°C

TILLER:	1 (Main culm)			2			Other		
	DW(g)	%P	mgP	DW	%P	mgP	DW	%P	mgP
Inflorescence	.259	.128	.332						
Flag leaf-blade	.096	.125	.120						
-sheath	.133	.122	.162						
Other leaves	.302	.101	.305				.067	.146	.098
Peduncle	.122	.129	.157						
Stem	.357	.032	.114						
Root	.431	.063	.272						

(c) Preanthesis temperature = 24/19°C

TILLER:	1 (Main culm)			2			3			4		
	DW(g)	%P	mgP	DW	%P	mgP	DW	%P	mgP	DW	%P	mgP
Inflorescence	.282	.164	.462									
Flag leaf-blade	.106	.188	.199									
-sheath	.128	.146	.187									
Other leaves	.280	.140	.392	.396	.150	.594	.320	.177	.566	.100	.148	.148
Peduncle	.100	.152	.152									
Stem	.291	.040	.116	.108	.108	.117	.095	.259	.246	.031	.166	.051
Root	.563	.104	.586									

(d) Preanthesis temperature = 30/25°C

TILLER:	1 (Main culm)			Other		
	DW(g)	%P	mgP	DW	%P	mgP
Inflorescence	.242	.403	.974			
Flag leaf-blade	.090	.389	.350			
-sheath	.090	.347	.312			
Other leaves	.242	.275	.666	.220	.272	.598
Peduncle	.055	.371	.204			
Stem	.165	.108	.178			
Root	.266	.211	.561			

Table App. 6.10 Intraplant distribution at maturity of dry weight, and phosphorus in low P plants following four preanthesis temperature regimes and grain development at 18/13°C (n = 3 plants).

a) Preanthesis temperature = 15/10°C

TILLER:	1 (Main culm)			2			Other		
	DW(g)	%P	mgP	DW	%P	mgP	DW	%P	mgP
Grain	1.021		1.42	.288	.165	.475			
Chaff	.395	.019	.075	.167	.034	.057			
Flag leaf-blade	.091	.021	.019						
-sheath	.146	.016	.023						
Other leaves	.250	.023	.058	.210	.120	.252	.287	.054	.154
Peduncle	.298	.035	.104						
Stem	.462	.012	.055	.247	.012	.030			
Root	.791	.042	.332						

(b) Preanthesis temperature = 18/13°C

TILLER:	1 (Main culm)			2			Other		
	DW(g)	%P	mgP	DW	%P	mgP	DW	%P	mgP
Grain	.964		1.692	.216	.296	.639			
Chaff	.384	.021	.081	.199	.054	.107			
Flag leaf-blade	.090	.023	.021						
-sheath	.126	.022	.028						
Other leaves	.205	.026	.053	.232	.018	.042	.194	.165	.320
Peduncle	.255	.034	.087						
Stem	.407	.013	.053	.252	.014	.035			
Root	.640	.049	.314						

(c) Preanthesis temperature = 24/19°C

TILLER:	1 (Main culm)			2			Other		
	DW(g)	%P	mgP	DW	%P	mgP	DW	%P	mgP
Grain	.786		1.675	1.155	.222	2.564			
Chaff	.355	.018	.064	.347	.042	.146			
Flag leaf-blade	.089	.023	.020						
-sheath	.121	.032	.039						
Other leaves	.215	.028	.060	.360	.021	.076	.211	.018	.038
Peduncle	.192	.051	.098						
Stem	.347	.015	.052	.479	.011	.053	.306	.018	.055
Root	.814	.067	.545						

(d) Preanthesis temperature = 30/25°C

TILLER:	1 (Main culm)			2			3			Other		
	DW(g)	%P	mgP	DW	%P	mgP	DW	%P	mgP	DW	%P	mgP
Grain	.937		2.705	.901	.242	2.180						
Chaff	.251	.036	.090	.464	.061	.283						
Flag leaf-blade	.052	.035	.018									
-sheath	.063	.024	.015									
Other leaves	.245	.048	.118	.110	.046	.051	.110	.052	.057	.326	.077	.251
Peduncle	.119	.029	.035									
Stem	.251	.013	.033	.422	.027	.114	.265	.024	.064			
Root	.551	.047	.259									

APPENDIX 7

Comparisons of phosphorus and nitrogen in senescing, senesced and dead leaf blades

The data below illustrate changes in total phosphorus and total nitrogen in senescing leaves.

The concentration of phosphorus at senescence was two to three times higher in the first leaves as in the flag leaf. This resulted in part of the change in the N:P ratio (Table APP. 7.1). As found in the experiment reported in Chapter 4, the grains gained weight after the flag leaf blade was completely senesced.

Comparison of N:P ratios in healthy and partly senesced tissues (Table APP. 7.2) indicated that the export of phosphorus precipitated a more rapid loss of nitrogen which was seen as a loss of chlorophyll from the leaf.

With the completion of senescence phosphorus was lost more rapidly than nitrogen in older leaves (leaves 1 and 2) but at the same rate as nitrogen in other leaves.

TABLE APP. 7.1 Phosphorus and nitrogen in just senesced and dead leaf blades of low P plants

Leaf number	Appearance	Plant growth stage	Days after sowing	Leaf dry wt.(mg)	Total phosphorus (%)	Total nitrogen (%)	N:P ratio
1	yellow[1]	flag leaf emerging	43	8.1 (0.3)[3]	0.062 (.006)	1.872 (.154)	30
2	"	flag leaf	46	13.3 (0.5)	0.048 (.002)	1.838 (.102)	38
3	"	ear in boot	50	27.6 (1.0)	0.038 (.001)	1.497 (.041)	39
4	"	ear fully emerged (anthesis - 3 days)	59	42.9 (1.5)	0.032 (.001)	1.169 (.035)	37
5	"	anthesis + 6 days	68	62.8 (2.3)	0.028 (.002)	0.945 (.036)	34
6	yellow	(anthesis + 24)	86	85.9 (1.6)	0.020 (.001)	0.784 (.016)	40
	dead[2]	(anthesis + 46)	108	77.2 (2.2)	0.017 (.001)	0.928 (.027)	55
7	yellow	(anthesis + 34)	95	105 (4)	0.022 (.001)	1.001 (.041)	46
(Flag)	dead[4]	(anthesis + 46)	108	96 (2.8)	0.018 (.001)	1.27 (.090)	73

1 when last chlorophyll is lost
2 at grain maturity stage
3 mean (± s.e.; n ± 12)
4 between days 95 and 108 the dry weight of central spikelet grains increased from 32.4 (±0.6)mg to 37.8 (±0.5) mg, n = 12.

TABLE APP. 7.2 Phosphorus and nitrogen in senescing leaf blades of low P plants[1]

	Healthy green[2] tissue (at base of blade)		Partly senesced tissue (mid leaf)		Senesced tissue (leaf tip)	
	% P[3]	N:P ratio[3]	% P	N:P	% P	N:P
Leaf number						
3	.098	34	.094	29	.049	35
4	.091	29	.093	25	.047	35
5	.073	37	.075	32	.030	29
6	.075	33	.073	29	.031	29
7(flag)	.094[4]	33	.085	32	.027	33

[1] 0.25 mMP supplied for 12 days

[2] Samples divided on appearance and oven dried

[3] Total nutrient levels in dry matter by autoanalyser (Chapter 2.7) each figure is the mean of two samples

[4] 0.177% at emergence.

APPENDIX 8: PLANTS GROWN IN SAND WITHOUT ADDITIONS OF PHOSPHORUS

Plants grown in the sand medium with the Nil P solution throughout were stunted and not all ears produced grain. These subsequently senesced and new tillers were formed as if the plant was vegetative rather than monocarpic. The grains which formed were found to contain an average of 0.1% total phosphorus.

Table. App. 8.1 Features of wheat (cv. Kite) grown in sand without additions of phosphorus

Height	30 cm
Total plant dry wt. (g)	0.4 to 1.1
Flag leaf area	2 to 3 cm^2
Tillers	1 to 2 green at any one time
Grains	0 to 12 per plant
weight (mg $grain^{-1}$)	30 ± 2
% P	0.21% ±.05
% N	2.97 ±.17
Harvest index (when grain(s) formed)	0.4

APPENDIX 9: OLIGOCULM WHEAT

Tillering and ontogeny in relation to daylength, temperature and vernalization

INTRODUCTION

The ideotype for a high yielding wheat, as proposed by Donald (1968), has a uniculm habit. Detillering experiments with normal tillering wheats growing in the field suggests that plants with two or three culms produces higher yields than plants with only one culm (Borojevic and Kraljevic-Balalic, 1980; Islam and Sedgley, 1981).

Future studies of the optimum tiller number per plant will probably utilize the gigas wheats selected by Atsmon and Jacobs (1977). Genotypes were found which either do not tiller (uniculm habit) or have a restricted tillering habit (oligoculm). However, expression of tillering in these wheats is under photothermoperiodic control.

When grown under long days with low night temperatures the uniculm selection No.494 produces only stunted plants - a new form of dwarfism (Inball, 1982). When grown at higher temperatures under long days 20% of plants are stunted, while under short days 25% of plants tiller (Atsmon and Jacobs, 1977).

My initial comparisons of a uniculm and an oligoculm wheat from Israel (obtained from the Australian Wheat Collection) indicated that the oligoculm was more robust (produced larger ears with more and larger grains, was often uniculm and was rarely stunted).

The thermo-photoperiodic conditions which control tillering in gigas wheats must be more clearly understood before plant breeders

and physiologists attempt to utilize gigas features. The studies reported here were conducted under strictly controlled temperatures and daylengths to obtain basic information on the factors which affect tillering in the oligoculm wheat.

MATERIALS AND METHODS

The oligoculm genotype 112-76 was obtained from the Australian Wheat Collection (AUS 20431). The seed stock was increased by growing plants in sand with the control nutrient solution (Appendix 1). This line was found to have a similar maturity to the cultivar Kite.

Experiment 1:

Two seeds were plants in perlite-vermiculite (50:50) in each 127 mm pot. For 2, 4 or 6 weeks the plants were grown in C-units within a phytotron glasshouse (Morse and Evans, 1962).

The temperature was 18° during the day (8 hours) and 13°C at night. Two photoperiods were maintained by providing 8 hours of natural light (mid-late summer) with or without an additional 8 hours of low intensity incandescent light. (These are referred to as long and short day treatments).

After 2, 4 or 6 weeks under these treatments the plants were moved to an open glasshouse. The temperature was 18°/13° and the natural daylight was supplemented with low intensity incandescent lamps to give a 16 hour photoperiod.

Experiment 2:

Germinated grains, which had been vernalized for 51 days at 2°C or not vernalized, were sown into sand and grown for 7 weeks under

the two photoperiods described above. There were two plants per pot. The temperature in the C-unit was 15°C by day and 10°C at night. The plants were then transferred to a glasshouse with natural light (late spring-summer), supplemented as above.

RESULTS

Data from experiments 1 and 2 are presented in Table App.9.1 and 9.2 respectively. The gigas features of these plants are shown in the number of grains per ear and the flag leaf area. Grain yield per each was high and ranged from 3.1 g, for plants grown for 6 weeks under short days, to 5.7 g for plants grown under long days. Details are not presented because shading possibly confounded grain filling in plants with different numbers of tillers.

The plants were strictly uniculm when grown under long days at 15°/10°C and partly uniculm under long days at 18/13 °C. When grown under short days, for as little as two weeks, the plants had 3 to 5 tillers. This would constitute an oligoculm habit because other bread wheats would produce 7 to 10 tillers under similar conditions (Chapter 7). Increasing the initial short day treatment from 2 weeks to 6 weeks delayed anthesis at 18/13°C by about 5 to 13 days relative to plants grown under long days. There were more fertile and sterile spikelets but fewer grains in the main culm ears of plants grown under short days for 4 or 6 weeks (Table App.9.1).

In experiment 2, at 15/10°C, vernalization reduced the time to anthesis by 3.8 days for plants grown under long days but had little effect on plants grown under short days. Under short days anthesis was delayed by 20 to 23 days, and significantly more fertile and sterile spikelets formed (Table App.9.2). (Grain numbers and grain weights are not yet available).

About 4-8% of the plants grown under long days were stunted; some died before elongating while others headed very late.

DISCUSSION

These experiments clearly demonstrate the thermo-photoperiodic sensitivity of the oligoculm gigas wheat when grown under controlled conditions. Short days, for as little as two weeks, caused the plants to tiller, delayed anthesis and led to more spikelets per ear. Long days at the lower temperatures of 15/10°C prevented tillering but caused a minor amount of stunting.

This is consistent with Inball's (1982) data for the thermo-photoperiodic behaviour of the uniculm line No.492. However, it appears that the oligoculm is less prone to stunting. A strict comparison of that genotype with the oligoculm used here is not possible because Inball's long day treatment was 20 hours and the temperature range 18/11°C. The low night temperature is believed to be responsible for stunting under long days.

These experiments demonstrate that the gigas oligoculm wheat could be of great value in studying the importance of tillering. By simply manipulating the photoperiod in the early seedling stage plants with or without tillers can be produced in genetically identical wheat (without laborious surgery which may have unwanted effects). For example this will reduce yield from:

yield = tillers x spikelets x grains per spikelet x grain dry weight

to

yield = spikelets x grains per spikelet x grain dry weight.

This flexibility in tiller production should also be useful for nutrition studies. It will reduce or eliminate the need to detiller plants grown under a high plane of nutrition.

In plants which do not tiller it will be possible to examine translocation of nutrient in a 'closed' plant system. Initially such studies will be conducted in controlled environments because the gigas wheats have performed poorly in the field. However, advanced material from crosses between local adapted genotypes and gigas wheats are showing promise in the field in Israel (Atsmon, personal communication). Merritt (1982) reported significant gains in harvest index in uniculm and oligoculm progeny of crosses between uniculm and multiculm wheats.

With an appreciation of the photo-thermoperiodic behaviour of gigas wheats further studies are now possible. Both plant breeding and selection for yield and basic studies of the nutrition and physiology of such wheats, should be most rewarding.

TABLE App.9.1: Data showing the effects of short and long days during early plant growth development at 18°/13°C on an oligoculm wheat

Treatment	Number of plants	Tiller plant^{-1} (at 6 weeks)[1]	Anthesis (days after sowing)[4]	Spikelets fertile	Spikelets sterile	Grains ear^{-1} (main culm)	Flag leaf area (cm^2)
Short days (8 hours sun)							
2 weeks	15	3.9(0.2)[2] [nil][3]	68.6(0.5)	19.1(0.4)	1.9(0.5)	81(3)	50(2)
4 weeks	16	4.6(0.3) [nil]	75.8(0.3)	18.5(0.3)	5.4(0.4)	61(4)	40(2)
6 weeks	28	4.1(0.3) [nil]	78.5(0.4)	21.0(0.4)	3.7(0.4)	67(3)	59(3)
Long days (8 hours sun + 8 hours incandescent lamps)							
2 weeks	16	1.8(0.1) [nil]	64.0(0.4)	17.9(0.4)	2.4(0.5)	86(3)	55(4)
4 weeks	14	1.9(0.1) [6%]	62.1(0.4)	16.9(0.5)	2.5(0.5)	84(3)	45(3)
6 weeks	15	1.9(0.1) [6%]	63.1(0.6)	18.1(0.6)	1.3(0.6)	86(3)	50(4)

1 also indicates ears plant^{-1} at maturity

2 standard error of mean

3 % plants showing stunting

4 anthesis was spread over 1-8 days in plants with 2 ears, and 5 to 8 days in plants grown under short days.

TABLE App.9.2: Data showing the effects of seed vernalization and daylength on plant growth and development at 15/10°C on an oligoculm wheat

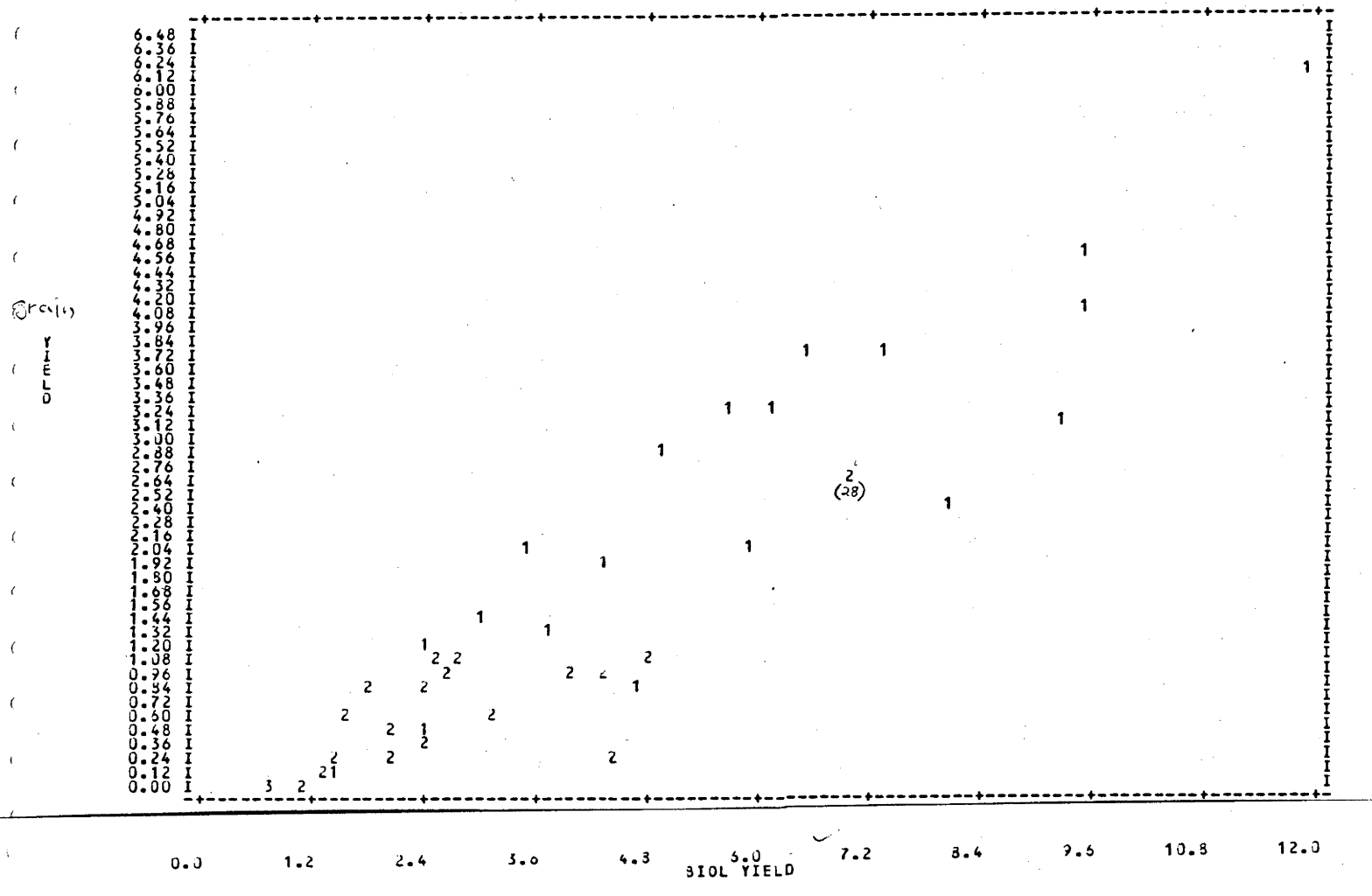
Treatment	Number of plants	Tillers plant^{-1} (at 7 weeks)	Stunted plants	Anthesis (days after planting)	Spikelets fertile	Spikelets sterile
Short days (8 hours sun for 7 weeks)[1]						
Not vernalized	24	3.6(0.2)	0	94.0(0.2)	20.2(0.5)	5.7(0.5)
Vernalized	24	3.1(0.1)	0	93.4(0.5)	20.0(0.4)	5.5(0.5)
Long days (8 hours sun + 8 hours incandescent lamp for 7 weeks)[1]						
Not vernalized	24	1.0(0)	2	73.9(0.4)	15.5(0.3)	2.5(0.4)
Vernalized	24	1.0(0)	1	70.1(0.4)	15.3(0.3)	1.5(0.4)

[1] after 7 weeks all plants were transferred to a glasshouse with natural light supplemented with incandescent lamps.

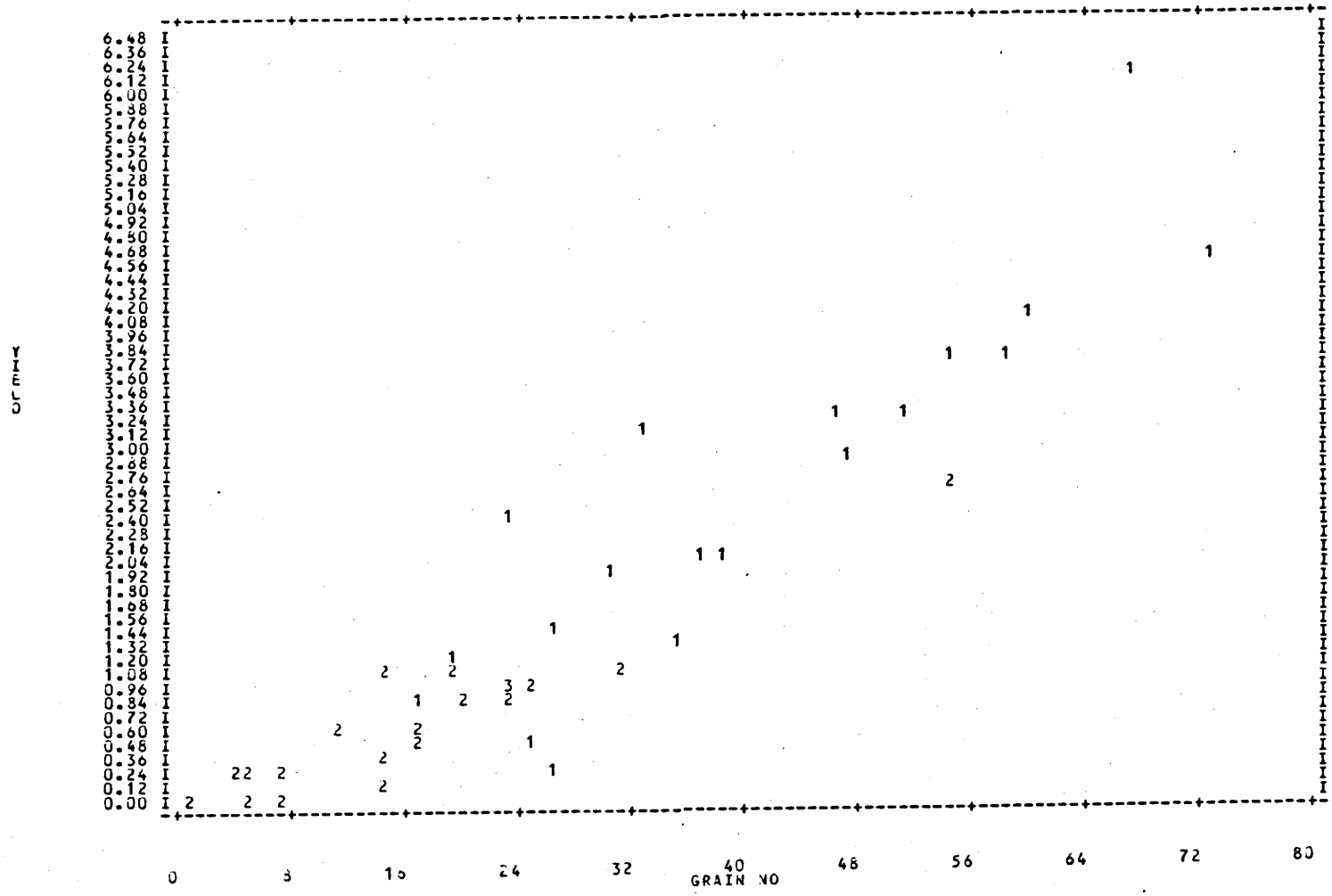
APPENDIX 10: GRAPHS OF RELATIONSHIPS BETWEEN PLANT PARAMETERS FOR 20 WHEAT GENOTYPES GROWN AT CONTRASTING PHOSPHORUS REGIMES

The following figures were plotted using the data in Table 7.2.

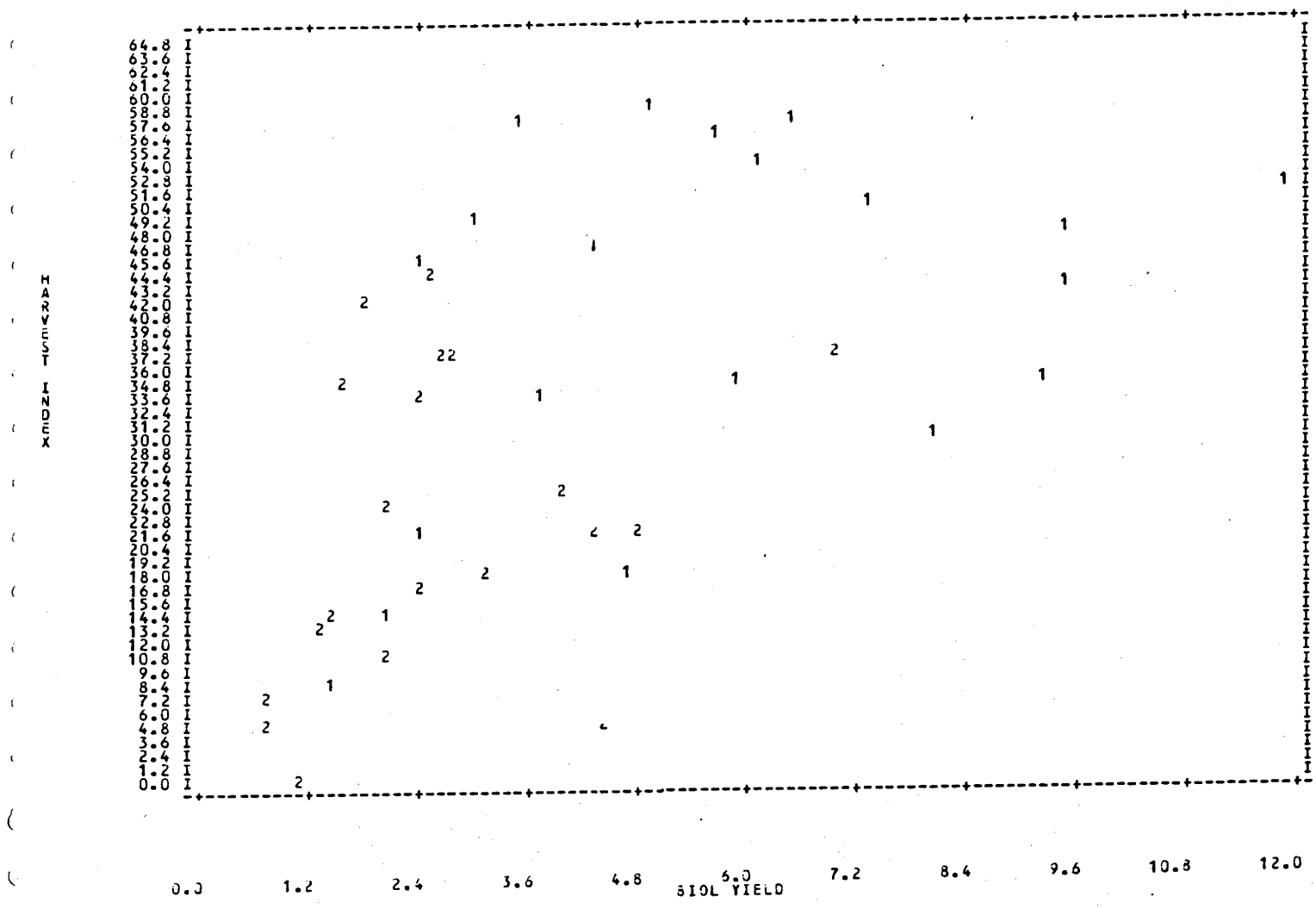
10.1	Grain yield	vs	Biological yield
10.2	Grain yield	vs	Harvest index
10.3	Grain yield	vs	Grains ear^{-1}
10.4	Harvest index	vs	Biological yield
10.5	Harvest index	vs	Grains ear^{-1}
10.6	Grain %P	vs	Grains ear^{-1}
10.7	Grain % P	vs	Average grain weight
10.8	Grain % P	vs	Grain yield



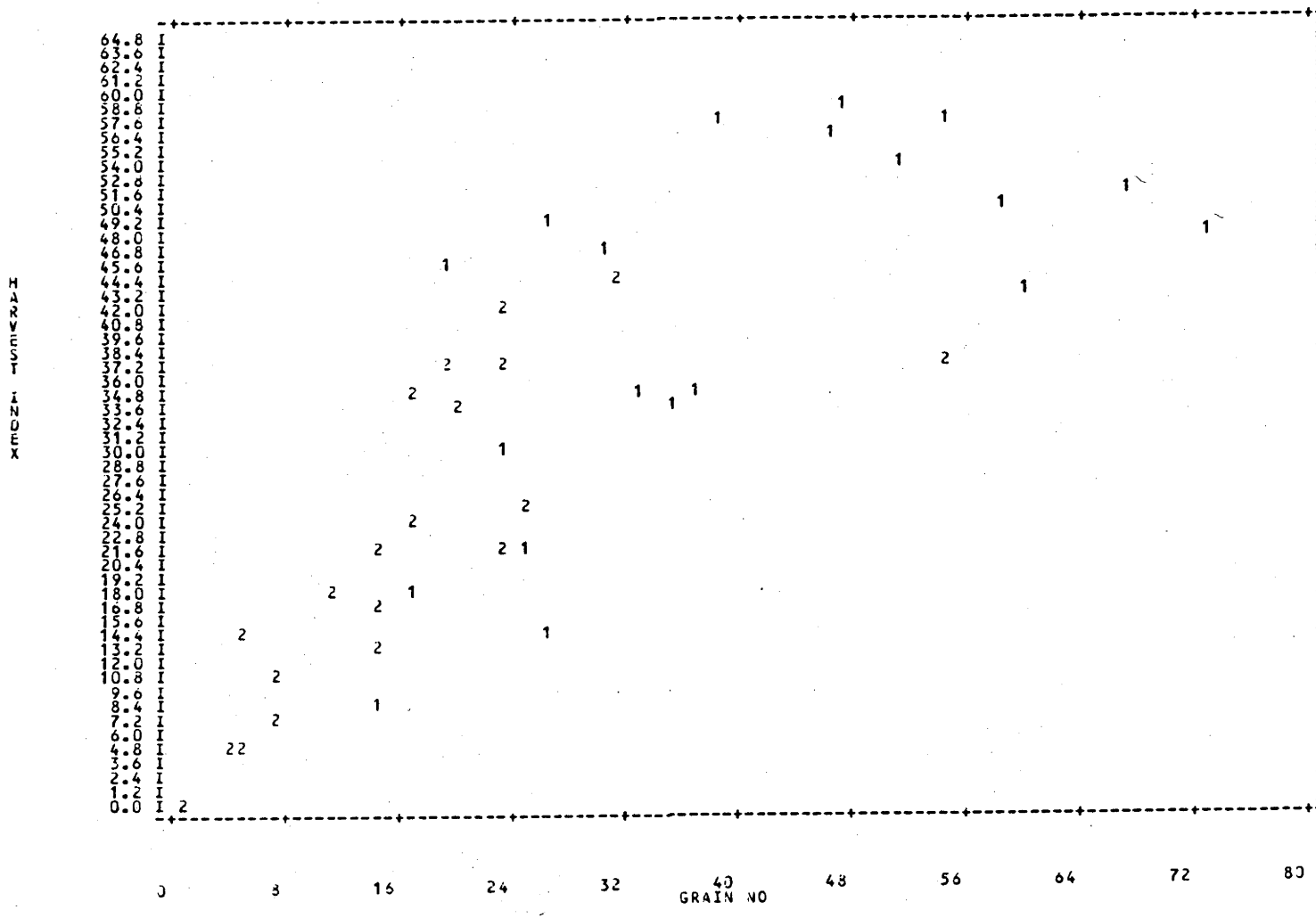
App. 10.1 Grain yield v's biological yield (g, main culm) for 20 wheats grown as control (1), and low P (2) plants.



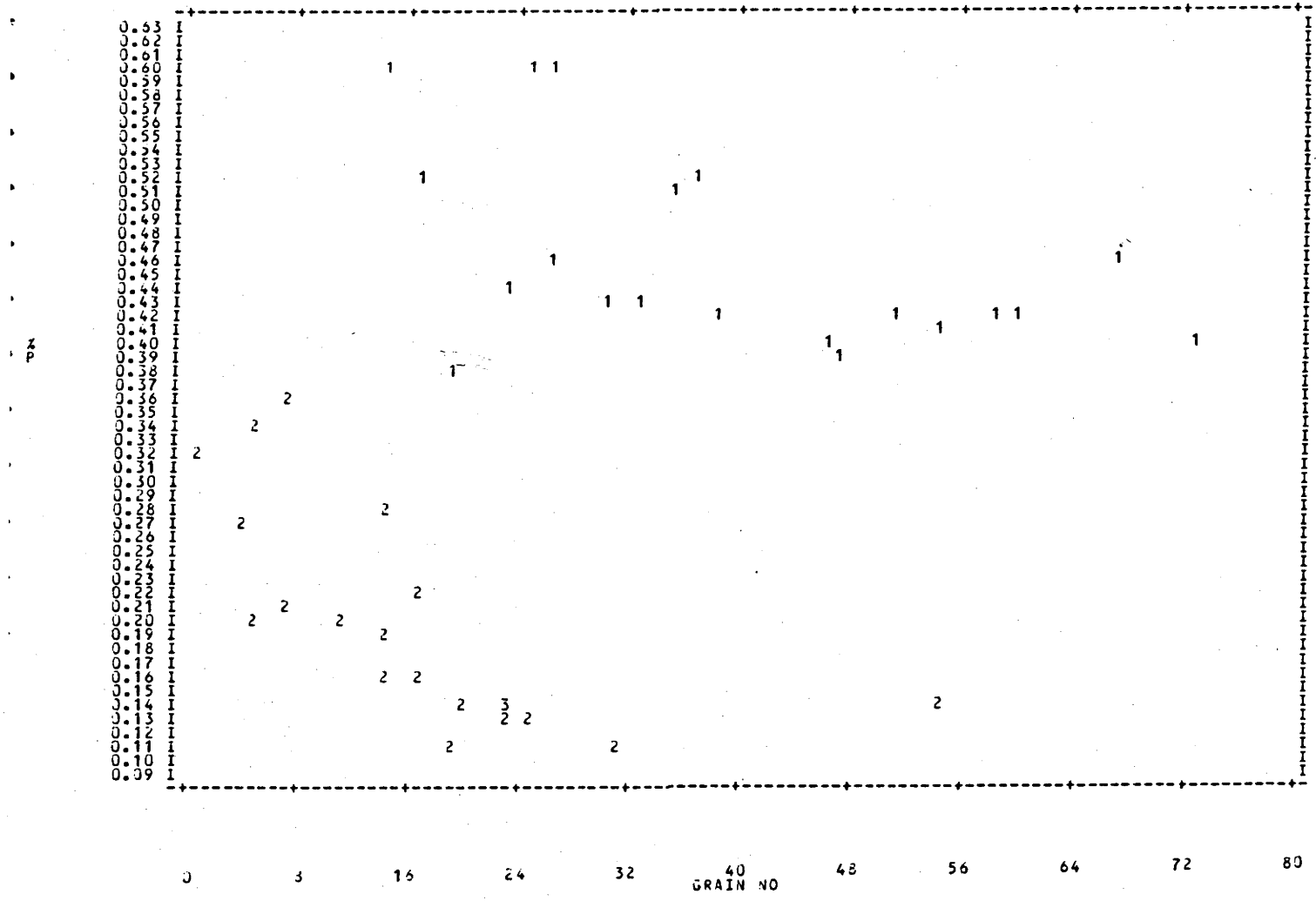
App. 10.3 Grain yield (g) v's grains ear^{-1} (main culm) for 20 wheats grown as control (1) or low P (2).



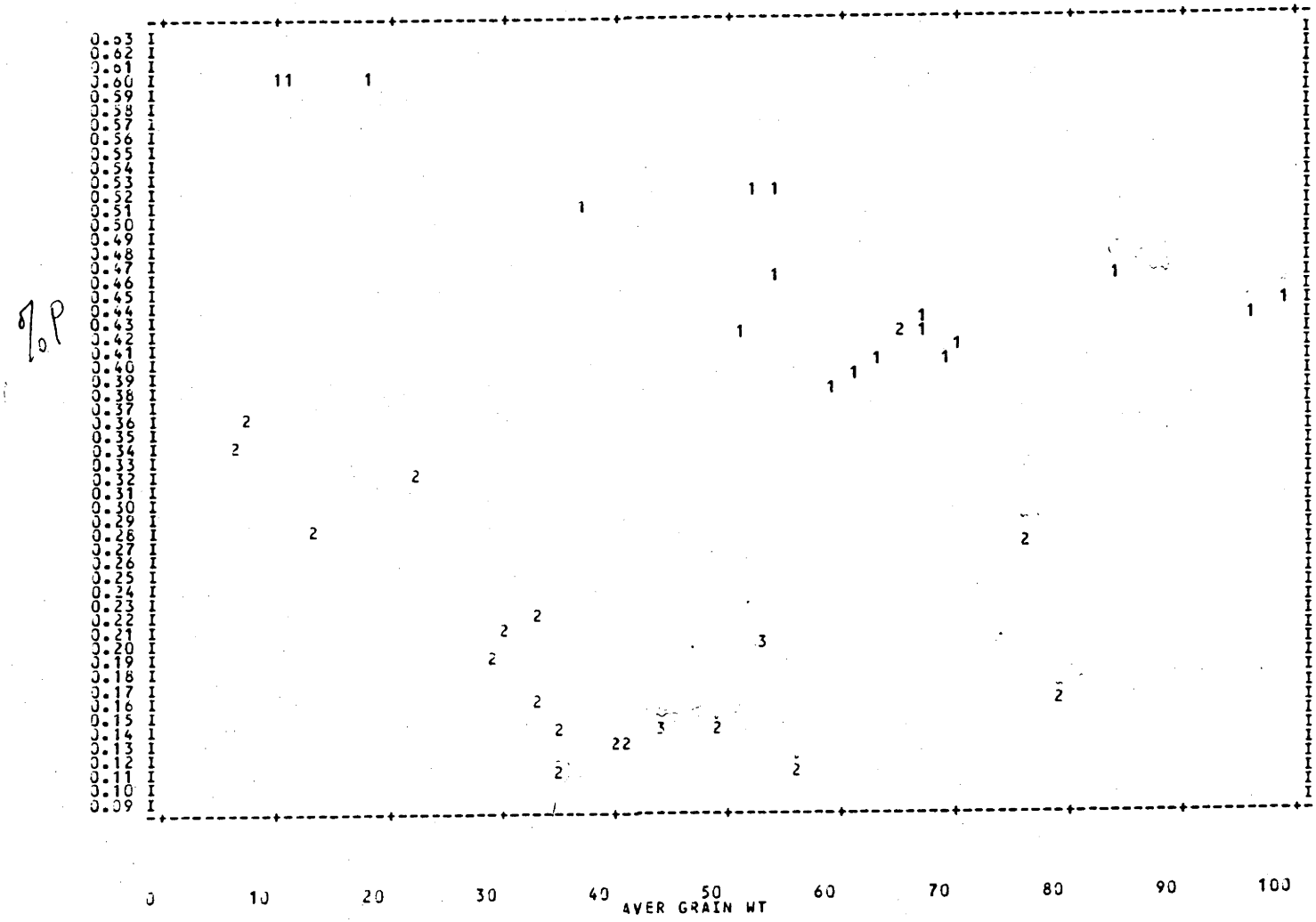
App. 10.4 Harvest index v's biological yield (g, main culm) for 20 wheats grown as control (1) or low P (2).



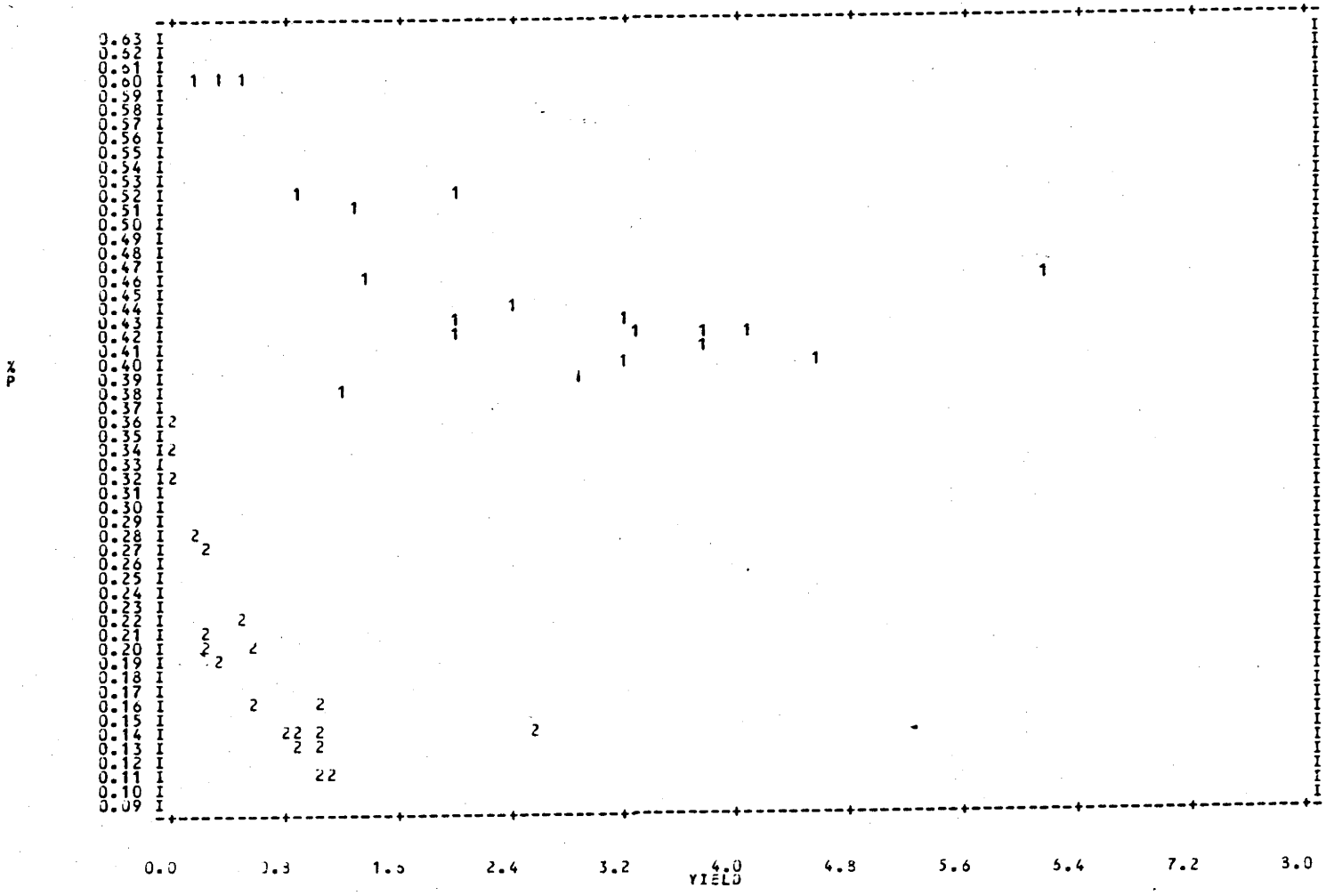
App. 10.5 Harvest index v's grains ear^{-1} (main culm) for 20 wheats grown as control (1) or low P (2).



App. 10.6 Grain phosphorus (% P) v's grains ear^{-1} (main culm) for 20 wheats grown as control (1) or low P (2).



App. 10.7 Grain phosphorus (% P) v's average mature grain weight (mg) for 20 wheats grown as control (1) or low P (2).



App. 10.8 Grain phosphorus (% P) v's grain yield (g, main culm) for 20 wheats grown as control (1) or low P (2).

a.

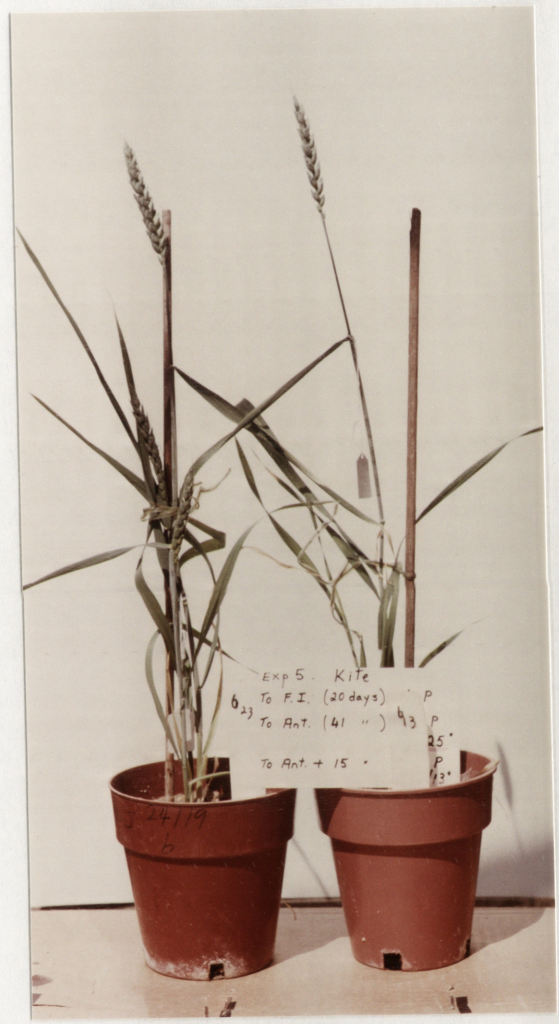
b.

1. Wheat (cv. Kite) 22 days after anthesis.

   a. 1 mM P b. 0.25 mM P supplied to (1-r) floral initiation, flag leaf emergence, anthesis and throughout growth.

   (Experiment 1 - Chapter 3).

a.



b.

2. Wheat (cv. Kite) 15 days after anthesis at 18/13° following a. 15°/10° or b. 30/25°C preanthesis. Both low P plants.

a.

b.

3. Wheat (Oligoculm 112-76)

a. Effect of long (left) and short days on plant growth and tillering (there are two plants per pot) at 18°/13°C. The long day plants are at anthesis.

b. Low P (left) and control plants grown after vernalization under long days at 15°/10°C. Only the head of the control plant has senesced.

(Chapters 3, 4 and 7 and Appendix 9).